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A NOTE ON THE INTERMEDIATE HOSTS OF THE TREMATODE, *CREPIDOSTOMUM COOPERI* HOPKINS, 1931, PARASITIC IN SPECKLED TROUT (*SALVELINUS FONTINALIS* (MITCHILL)) IN SOME LAKES AND RIVERS OF THE QUEBEC LAURENTIDE PARK[1]

BY L. P. E. CHOQUETTE[2]

Abstract

In some areas of the Quebec Laurentide Park the developing larval stages of the trematode *Crepidostomum cooperi* were found in sphaeriid clams of the genus *Pisidium* (*P. subtruncatum*, *P. compressum*, *P. adbitum*, *P. lilljeborgi*, and *P. nitidum*), and the encysted state in nymphs of the mayfly *Hexagenia recurvata* and in those of another species of mayfly of the genus *Polymitarcys*. These constitute new records of intermediate hosts for *C. cooperi*. Sexually mature worms were recovered from trout three to four weeks after the trout fed upon infected mayfly nymphs, thus confirming Hopkins' observation on the length of time required by the worm to attain sexual maturity.

As shown previously by the writer (5) *Crepidostomum cooperi* Hopkins, 1931, is common in speckled trout (*Salvelinus fontinalis* (Mitchill)) throughout the Laurentide Park in the Province of Quebec. The author has also found this parasite in trout from lakes of the Mont Tremblant Park area and in Argenteuil, Labelle, St. Maurice, and Champlain counties in this province. During the summers of 1952 and 1953 attempts were made to determine the intermediate hosts of *C. cooperi* in lakes and rivers of the Chateau Beaumont territory in the Laurentide Park.

Historical Review

The life history of *C. cooperi* has been studied by Hopkins (10) who also described the morphology of the various larval stages and the adult. Brown in 1927 (4), and Crawford, in 1943 (7), reported on the life history of *Crepidostomum farionis*, while Ameel in 1937 (2) and Henderson, in 1938 (8), reported on that of *C. cornutum*. As shown by Brown and Hopkins, the redial and cercarial stages of species of *Crepidostomum* are to be found in sphaeriid clams,

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while the metacercarial stage has been shown by Cooper (6), Noller, Faust (Hopkins (10)), Brown (4), Baylis (3), Abermathy (1), Ameel (2), Henderson (8), and Crawford (7), to occur in a variety of aquatic arthropods.

Intermediary Molluscan Hosts

In his study on the life history of *Crepidostomum cooperi*, Hopkins (9) found the redial and cercarial stages of the fluke in the sphaeriid, *Musculium transversum* (Say), in various localities in the United States. He also recorded the infection in a single specimen of a species of the genus *Pisidium*.

In the present study the following species of clams collected in Lake Turgeon, Laurentide Park, harbored the larval parasite: *Pisidium subtruncatum*, *P. compressum*, *P. adbitum*, *P. llijeborgi*, and *P. nitidum*. In all cases the rate of infection was low, being 2 to 3%. Unfortunately, because of climatic conditions prevailing in the Laurentide Park early in the fall and lasting until late spring, it has been impossible to collect clams during that period in order to determine whether the low incidence is due to a seasonal variation or related to ecological or other factors.

Intermediary Aquatic Arthropod Hosts

In their studies both Brown (4) and Hopkins (10) have shown that mayfly nymphs are the second intermediate hosts of *Crepidostomum farionis*, *C. cooperi*, and *C. isostomum*. Earlier, in 1915, Cooper (6) reported finding encysted larvae in nymphs of ephemerids of the genus *Hexagenia*. Cooper believed these larvae to be those of *C. laureatum* which Hopkins (9) subsequently considered to consist of two species which he described as *C. cooperi* and *C. canadense*. Cooper's record of metacercaria in *Hexagenia* nymphs is the earliest one on the developmental stages of species of *Crepidostomum*; it is the only one in Canada.

In his study on the biology of *C. cooperi* Hopkins (10) reported finding the metacercaria encysted in the muscle and body cavity of the nymph of the mayfly, *Hexagenia limbata* (Guerin). He found the rate of infection in the nymphs collected during the summer months to vary between 61 and 95%. According to Hopkins there is no apparent seasonal variation in the rate of infection. During the present study, the metacercarial stage of *C. cooperi* was found in the nymphs of the mayfly *Hexagenia recurvata* (Morgan), and in the nymph of a species of the genus *Polymitarcys* collected in Grand Lac Carré, Laurentide Park. In the first species over 80% of the nymphs examined harbored the larval parasite, in the second, about 20%. Other aquatic arthropods, including dragonfly nymphs, damselfly naiads, an *Gammarus pulex*, all very common in Grand Lac Carré, were examined, but were negative.

Twelve parasite-free, two-year-old, hatchery-raised speckled trout were fed nymphs of *Hexagenia recurvata* and nymphs of *Polymitarcys* sp. collected from Grand Lac Carré and the Ste. Anne du Nord River. In both cases, infection

was successful, and Hopkins' (10) finding that the time required by the worm to develop into sexually mature adults varied between three and four weeks was confirmed.

Acknowledgments

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MORPHOLOGY OF THE FACE IN THE HYMENOPTERA[1]

By R. S. Bigelow[2]

Abstract

Facial structure was compared in over 925 of the 1,945 Nearctic genera and subgenera of the order Hymenoptera. The clypeus is considered as the facial region between the labrum and a definite system of strengthening inflections associated with the tentorium. The primitive position of the anterior tentorial pits was probably at the level of the ventral edges of the genae. In many of the species examined they lie well above the ventral edges of the genae. This modification was probably produced by ventral extension of the genae, and not by dorsal migration of the clypeus. In many species, however, the pits lie at the ventral rim of the face. This modification was probably produced first by enclosure of the clypeus between ventral extensions of the genae, and then by ventral migration of the tentorial pits. The formation of new sclerites by the secretion of additional cuticular material was observed and described in several groups. Similarities in facial structure between groups widely separated by taxonomists are pointed out.

This study was undertaken in an attempt to test the validity of existing concepts of the morphology of the face in the Hymenoptera and to increase our understanding of the differences in facial structure that exist within this group of insects. Facial structure was compared in over 1000 genera and subgenera, chiefly from specimens in the Canadian National Collection, Ottawa, Canada.

Since the point of view from which this study was made differs somewhat from those of other published works on the subject, the principal differences will be discussed briefly at the outset.

Many insect morphologists have ignored the role of function in evolutionary change, and some have implied that the consideration of function is actually detrimental to morphological work. This study, however, was made on the assumption that most structural changes cannot be understood adequately unless they are considered simultaneously with function.

Very little fossil evidence is available to students of insect morphology, and consequently there is a great gap in our knowledge of the ancestral beginnings of insects. Admirable attempts have been made to fill this gap through studies of existing forms, and some progress has been achieved. Nevertheless, our knowledge of early ancestral conditions has remained, of necessity, hypothetical and obscure. Most morphological studies have been based on the assumption that primitive conditions are known, or at least on the assumption that, if certain conditions can be considered primitive, then a certain series of changes may have taken place. This is understandable, since we must begin somewhere, and the logical place to begin is at the beginning. This is often a dangerous starting point in insect morphology, however, for the beginnings are usually unknown. The point of view adopted here is that science must proceed from the known toward the unknown, not from the

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unknown to the known. We know, or can know, the structural conditions in many thousands of insect species, and from these conditions it should be possible, through a comparison of differences within well-defined groups, to attain an understanding of the more recent evolutionary changes. When these more recent changes are sufficiently well understood, it may be possible to deduce less recent changes, through which the well-defined existing groups originally diverged from a common ancestral stock. Until existing differences are more fully understood, however, it is held that "phylogenetic trees" involving many distantly related groups cannot be inferred on a sound, scientific basis.

For these reasons, the facial structure in the Hymenoptera was studied as it exists today and an attempt was made to draw conclusions from the existing differences. Only two assumptions were made as to conditions probably present in ancestral Hymenoptera. It was assumed that the structure in sawflies has retained more of the ancestral features than has that in most other hymenopteran groups; and that an "orthopteroid" facial structure, in which the clypeus projects as a free lobe below the ventral edges of the genae, was present in ancestral Hymenoptera. These assumptions are considered valid by virtually all entomologists and are supported by the scanty fossil evidence that is available. Apart from these assumptions, conclusions were drawn from the structural differences studied.

Most of the difficulties encountered in defining the facial region called the clypeus are derived from the immense diversity in the facial structure of existing insects. The definitions of the clypeus given in textbooks apply to conditions in generalized insects, and are often difficult to apply to the more specialized forms that make up the majority of the class Insecta.

Another difficulty is derived from a lack of agreement as to whether the existing clypeus should be considered as a remnant of a primitive body segment, or a primitive sclerite which has retained a morphological individuality by means of which it can be distinguished from a neighboring sclerite, the frons. Ferris (3) considered the clypeus as a remnant of a primitive body segment. Snodgrass (6, 7) defined the clypeus by the attachment of the dilator muscles of the cibarium on its inner surface, and the frons by the origin of the labral muscles on its inner surface. DuPorte (1) and DuPorte and Bigelow (2) defined the clypeus primarily as a preoral structure, anterior or ventral to the primitive mouth opening.

Ferris' assumption that the integumental grooves on the insect head represent primitive intersegmental lines, and have persisted as such despite the profound functional and structural changes that have taken place in the many millions of years since the insect head became a composite structure, is not convincing and has not been generally accepted.

Snodgrass' definition applies more to muscle attachments, and that of DuPorte and Bigelow more to the position of the primitive mouth opening, than to integumental features of the face. It is often difficult or impossible to determine the position of muscle attachments, or of the mouth opening, in

dried specimens, and existing positions of these two characters are not necessarily primitive. By a study of the facial integument in terms of muscle attachments, or of the mouth opening, it might be possible to obtain an understanding of evolutionary changes in muscle attachments, or of the mouth, but since there is apparently no mechanism whereby the interrelationships between the integument and these other organs must remain fixed and immutable, such a study would not necessarily provide an understanding of integumental changes. Therefore, for the purposes of this paper, facial structure was studied in terms of differences in the interrelationships between the anterior mandibular condyles, the anterior tentorial pits, and the system of strengthening integumental inflections usually associated with these structures. The clypeus is correspondingly regarded as that portion of the integument between the labrum and this system of strengthening inflections. This definition happens to correspond, in the Hymenoptera, with that of Snodgrass, that of DuPorte and Bigelow, and with the region of the face called the clypeus by taxonomists. It differs from previous definitions in that the essential feature of the hymenopteran clypeus is regarded as its position below and between a system of functional integumental inflections.

The insect tentorium forms an internal framework that provides ridigity to the otherwise more or less unsupported ventral region of the head. It also provides attachment for muscles of the mandibles, maxillae, labium, hypopharynx, stomodaeum, and antennae. It therefore not only braces the ventral region of the head, but also provides a rigid base from which motion can be transmitted to the mouth parts, fore-gut, and antennae. The anterior tentorial pits mark externally the points at which the anterior tentorial arms are invaginated from the integument of the face. The stresses applied to the tentorial arms by the action of the internal muscles associated with them are transmitted, therefore, to the facial integument between the anterior tentorial pits, and this probably explains the widespread presence of a strengthening inflection, usually marked externally by the frontoclypeal sulcus, between the tentorial pits. The anterior mandibular articulations are almost always associated with the anterior tentorial arms, either directly through short ventral protuberances from the latter, which are also continuous with the integument of the clypeus, or less directly through the medium of distinct internal ridges (clypeogenal inflections) extending from the mandibular epicondyles to the tentorial arms and entirely continuous with the latter (see Fig. 20). These internal inflections are usually marked on the exterior surface of the integument by distinct grooves or sutures.

The system of grooves extending from one anterior mandibular articulation to the other and passing through the anterior tentorial pits is called the *epistomal suture*. One of the most consistent interrelationships among the facial structures in the Hymenoptera is that between the anterior tentorial arms and the anterior mandibular articulations. In the vast majority of species the mandibular epicondyles and tentorial arms are united either more or less directly or through internal ridges as outlined above. This can be

explained on the assumption that the tentorial arms provide the most rigid fulcrum, or base for the fulcrum, against which the anterior mandibular articulations must get purchase or support. The epistomal suture, then, is regarded as a functional supporting structure associated with the anterior tentorial arms and the anterior mandibular articulations; and the clypeus, as that region of the integument between and below the epistomal suture and above the labrum.

In generalized insects, and in some Hymenoptera (e.g. *Pleroneura*, Fig. 2), the anterior tentorial pits lie near the anterior mandibular articulations at the level of the ventral edges of the genae, and the clypeus projects ventrally as a free lobe. In many Hymenoptera, however, the tentorial pits lie well above the mandibular articulations and the ventral edges of the genae, and the free ventral extent of the clypeus is reduced (e.g. *Apis mellifera*, Fig. 19). Snodgrass (6, 7) has proposed that the transition from the former to the latter condition was effected first by an "upward arching" of the epistomal suture and then by a dorsal migration of the tentorial pits along this suture to their position in the honey bee (Fig. 19). DuPorte (1) questioned this explanation on the grounds that the tentorial pits are not likely to follow the migrations of a secondary integumental inflection such as the epistomal suture. DuPorte and Bigelow (2) proposed an alternative explanation, based on the assumption that the tentorial pits remained relatively stationary while the genae were extended ventrally on either side of the clypeus. This latter proposal will be discussed somewhat more fully here.

Any shift in the positions of the anterior tentorial pits would involve a shift in the orientation of the anterior tentorial arms, and would thus require a rather complex readjustment of the internal organs (e.g. muscles) associated with these arms. There is no doubt that such complex structural changes have taken place, but in this instance it is simpler to assume that the genae have been extended ventrally. There can be no doubt that ventral extensions of the genae have occurred in the Hymenoptera. Conditions in the braconid genera *Agathis* and *Cremnops* (Figs. 7–10) can be interpreted in no other way, and such conditions recur in the ichneumonoid genera *Coccygomimus*, *Hoplismenus*, *Pristomerus*, and *Cremastus*, as well as in the apoid genus *Bombus*. Conditions in many sawfly species (e.g. Figs. 3, 4, 5, 6) are apparently incipient stages in a ventral extension of the genae; it is at least as reasonable to assume this as to assume that the tentorial pits have migrated upward.

Possible selective advantages of ventral extension of the genae would be increased space for ventral enlargement of the eyes and increased space for the development of accessory pharyngeal organs. The unusually large eyes in many Hymenoptera suggests that enlargement of the eyes may in many cases have been the basic cause of a ventral extension of the genal area. In other species (e.g. *Cremnops*, Fig. 10) the genae have been extended far below the ventral extremities of the eyes, and therefore enlargement of the eyes is not the only factor involved in this modification.

Not every structural change confers an additional selective advantage, and some animal structures are known to be distinctly disadvantageous. Disadvantageous structural features may evolve through genetic association of these with the evolution of other, advantageous characters. Therefore, morphological explanations cannot be declared invalid merely on the grounds that they involve apparently disadvantageous changes. However, there is a large body of evidence in support of the view that the over-all pattern of evolution is adaptive. Even where altered conditions of life have rendered formerly adaptive characters disadvantageous, subsequent changes in such characters are usually adaptive in the sense that the disadvantageous characters are usually reduced or transformed into neutral or advantageous characters.

In a choice between two possible explanations of a given structural change, one of which involves an apparent selective disadvantage and the other an apparent advantage, it is held here that the latter should be favored as more likely until definite evidence to the contrary is available.

Separation of the tentorial pits and mandibular articulations creates the need for an auxiliary structure to retain the rigidity against mandibular action that was originally provided more or less directly by the anterior tentorial arms. This has been accomplished in the Hymenoptera by the anatomical development of elongations from the ventral portions of the tentorial arms at the point where the latter meet the face (Fig. 20, *c.g.i.*). These extensions from the tentorial arms (i.e. clypeogenal inflections) form braces between the tentorial arms and the mandibular articulations. Their development can be seen very clearly in sawflies (Figs. 3, 4, 5). In some sawfly species they can be seen externally as well as internally, and they are always completely continuous with the tentorial arms. The clypeogenal inflections are extensive in bees, sphecoids, and vespoids, where the tentorial arms are almost horizontal in orientation, and they apparently provide as efficient a brace against mandibular action as was perhaps originally provided directly by the tentorial arms. The selective disadvantage that was probably involved in the separation of the tentorial arms and mandibular articulations was apparently offset by the greater advantage of increased genal area, and the end result was the efficient structural design in bees, wasps, and sphecoids.

Snodgrass (6) described the tentorium of generalized insects as "a horizontal, X-shaped brace between the lower edges of the cranial walls". This implies that the hymenopteran tentorium was also horizontal before the clypeus was enclosed between the genae. If it is assumed, then, with Snodgrass, that in the honeybee the tentorial pits have migrated some distance above their primitive position, it is reasonable to expect the tentorial arms in the honeybee to be tilted upward from the occipital foramen to the anterior edge of the face. It can be seen in Fig. 20, however, that this is not the case. They are tilted distinctly downward. If the pits have migrated dorsally in this case, it follows that the occipital foramen must also have migrated dorsally, and that the dorsal portions of the cranium were also extended dorsally. By this

reasoning, the entire head, except for the ventral rim, has been extended dorsally. It is simpler to assume that the ventral rim of the head has been extended ventrally.

There is considerable evidence in the Hymenoptera suggesting a ventral migration of the tentorial arms from an originally horizontal position, but there is no evidence of a similar dorsal migration, even where the tentorial pits lie well above the mandibles as in Fig. 20. The tentoria of many species, including representatives from all superfamilies, were examined in the course of this study, and in every case the course of the anterior tentorial arms was anterior and ventral from the occipital foramen to the tentorial pits.

What the actual orientation of the tentorial arms may have been in ancestral Hymenoptera can only be surmised. It was probably also variable in those ancestors. In any case, dorsal migration of the pits would involve dorsal migrations of the tentorial arms. Although dorsal migration of the pits would confer no apparent advantage, ventral migration would tend to regain the original close contact between the anterior arms and the mandibular articulations.

A tendency for the reduction of the clypeus is almost as widespread in the Hymenoptera as is the tendency for its enclosure between the genae. In most chalcidoids, and in many other species, the tentorial pits lie at the extreme ventral edge of the face, and no clypeal lobe extends beyond this level. This reduction of the clypeal region could be explained on the assumption that the free distal extremities of the clypeus have merely been lost. If this is accepted, it must also be assumed that the occipital foramen has been shifted dorsally to a great extent in order to explain the very marked anteroventral orientation of the tentorial arms in typical chalcidoids (Fig. 21). Ventral closure of the occipital foramen, by ventral extension of the postgenal areas, is known to have taken place in the Hymenoptera. The resulting structure is called the *hypostomal bridge* by Snodgrass (7) and the *genaponta* by Ross (5). Where the postgenae have been extended ventrally to a greater extent than the genae, the head may tend to be "tilted forward", and the clypeal region may appear to have migrated ventrally. This has undoubtedly taken place in many Hymenoptera, and probably is one of the underlying causes of the tentorial orientation in chalcidoids. However, it is also probable that the genae as well as the postgenae have been extended ventrally in chalcidoids and, if so, a ventral migration of the tentorial arms may be assumed in order to explain the position of the tentorial pits at the ventral rim of the face and the absence of a free distal extremity of the clypeus.

It would appear that, in chalcidoid types, the clypeus was reduced by ventral and mesal extensions of the frons and genae above and beside it. That tendencies toward genal encroachments on the clypeal area exist in chalcidoid types is evidenced by the marked genal encroachments present in some pteromalid and cynipoid species, in which the striated genal areas shown in Fig. 16 extend mesally across the clypeal area and even meet on the mid-line in some cases. Frontal encroachments on the clypeal area are present in

chrysidoids, ants, sphecoids, and others. It is not unlikely that they have occurred also in chalcidoids. Ventral migration of the entire clypeal area is easily explained on the grounds that the integument is increased in extent above, rather than below, the tentorial pits.

If the clypeus has been reduced in chalcidoids by both frontal and genal encroachments, it must previously have been enclosed between the genae. That this was probably the case is witnessed by the extreme anteroventral orientation of the tentorial arms; the presence of an "enclosed" clypeus in *Perilampus*, in pteromalids (Fig. 16), and in *Leucospis*; the presence of an "enclosed" clypeus in the more generalized sawflies; and the fact that the minute size and parasitic way of life of chalcidoids are obviously specializations.

It is reasonable to conclude that the clypeus was enclosed between the genae in ancestral chalcidoids, and that subsequently it has been reduced from above. This sequence probably has been followed in many other Hymenoptera as well, as witnessed by the widespread tendency for the antennal foramina to encroach on the clypeus, and even for the clypeus and antennae to be underfolded to such an extent as to be invisible in the anterior aspect, as in orussids.

The presence of additional cuticular material, differing distinctly in color, texture, and transparency from the surrounding integument, was observed in many species of the Tenthredinidae, Siricidae, Ichneumonidae, Chalcidoidea, Cynipoidea, and Proctotrupoidea. All intermediate stages between almost complete transparency and complete opacity of this cuticular material are present in different specimens of single sawfly species such as *Ametastegia inornata* (Say), *Aphilodyctium fidum* (Cresson), *Allantus cinctus* (L), and others. Differences were observed between opposite sides of the clypeus in single specimens of *Eriocampa juglandis* (Fitch) and *Hemichroa crocea* (Fourcroy). In one specimen of *Hemichroa crocea* the cuticular material between the clypeus and genae was semitransparent. After this specimen was boiled in caustic potash and left overnight in water, this same material became jet black and absolutely opaque. It was still clearly distinguishable, however, since it was more smooth and shiny than adjoining parts of the clypeus and genae. It had, in fact, become identical with conditions present in many pinned specimens. Similar conditions exist in rhyssine ichneumonids, chalcidoids, cynipoids, and others, and there can be little doubt that the resulting sclerites were formed relatively rapidly, and at a late stage of pupal development.

In the pimpline ichneumonid *Pseudorhyssa sternata* (Fig. 14), the limits of a typical pimpline clypeus can be seen distinctly beneath transparent cuticular material in some specimens, whereas in other specimens these limits are completely obscured beneath a hard, opaque *clypeogenal bridge* (*B*). Similar conditions are present in *Centeterus tuberculifrons* (Fig. 15), where two distinctly different regions are present in the clypeus. The boundary between these regions is indicated by the dotted line in Fig. 15. The dorsal and central region (Fig. 15, *C*) protrudes above the lateral and ventral regions (Fig. 15, *B*), and the line of separation between the two is distinct. The

integument of the central region is indistinguishable in texture, color, etc., from the frons above it, while the lateral regions are much more smooth, and very similar in color and texture to the cuticular material previously described in the Tenthredinidae and rhyssine ichneumonids. The central region of the clypeus in *C. tuberculifrons* is similar in shape and position to the clypeus in many species from closely related groups, and the lateral portions have apparently been added by cuticular secretion. In the proctotrupoid families Evaniidae, Gasteruptiidae, and Proctotrupidae extensive triangular sclerites are present above the mandibular articulations and between the clypeus and genae. These sclerites differ in color and texture from the surrounding integument in some specimens and probably were formed by cuticular secretion in the same manner as the clypeogenal bridges discussed above.

It is clear that these clypeogenal bridges serve to cement and support the edges of the clypeus and genae when the latter have descended below the original position of the mandibular articulations.

In the Pteromalidae and Cynipoidea the entire clypeal area has been overlaid with similar cuticular material (e.g. Figs. 16-18). In some species this material has become opaque, and the clypeus and tentorial pits are completely hidden beneath it. Such species often superficially resemble typical chalcidoids in facial structure, in that the clypeus and tentorial pits cannot be seen in the anterior aspect. Internal examination, however, reveals an essential difference, in that the tentorial arms in pteromalids and cynipoids meet the face some distance above the ventral edge, whereas in typical chalcidoids they are usually at the extreme ventral edge.

Cuticular secretions of this kind are not difficult to understand. They are merely continuations of the normal process of cuticle formation. The entire sclerotized integument, in fact, is formed in much the same way. It is interesting that, before the genetic mechanisms underlying the formation of previous structures has been altered, this tendency for cuticular additions has appeared. This is in accord with the view that initial adjustments to new needs will be made in the simplest manner possible, and that more fundamental changes, involving greater readjustments of the genetic constitution, will tend to occur more slowly. When the genetic constitution has become fully adjusted to the new conditions, "pioneer" devices like the cuticular secretions in question will become indistinguishably blended into the normal process of development, i.e., traces of the old structural configuration in adults will tend to disappear.

The fact that new sclerites can be created and sutures completely hidden through the very simple and rapid means of additional cuticular secretions has important implications. Sclerites and integumental grooves may not be homologous, even in relatively closely related species, and should, therefore, be used only with extreme caution in the comparison of distantly related groups.

In the course of this study, similarities in facial structure were noticed between hymenopteran species that are widely separated by taxonomists, as

indicated by Muesebeck *et al.* (4). These similarities must be due either to convergent evolution, or to unnatural arrangement in this catalogue. Convergence, in the sense used here, means the independent development of superficially similar structures in different evolving lines. If the common ancestors of two species did not possess a structural feature that is now common to both, the similarity is the result of convergent evolution. If the common ancestors of two species did possess a character now present in both species, the similarity of the character in the two species is the result of parallel evolution. Convergent similarities usually suggest a closer relationship than actually exists. Parallel similarities are usually more indicative of near relationship than are convergent similarities. It is important, both in taxonomy and morphology, to consider whether or not a given similarity is convergent or parallel before using it as a criterion of close relationship.

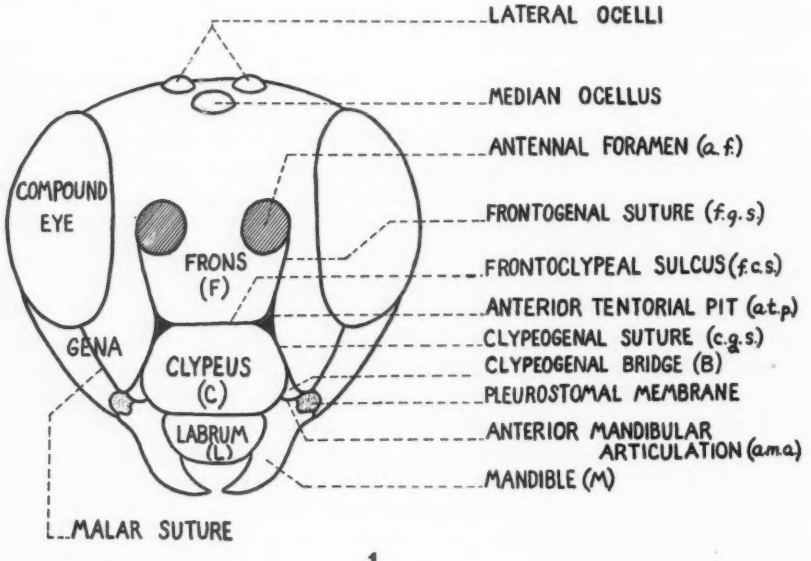

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FIG. 1. Diagram of anterior aspect of hymenopterous face.

EXPLANATION OF FIGURES

The following illustrations were selected from those included in a Ph.D. thesis. Unless otherwise specified the figures represent the anterior aspect of the face.

ABBREVIATIONS IN FIGS. 1-21.

A—integumental apophysis.
a.a.t.—anterior tentorial arm.
a.f.—antennal foramen.
a.m.a.—anterior mandibular articulation.
a.t.p.—anterior tentorial pit.
B—clypeogenal bridge.
C—clypeus.
c.g.i.—clypeogenal inflection.
L—labrum.
M—mandible.
p.m.a.—posterior mandibular articulation.

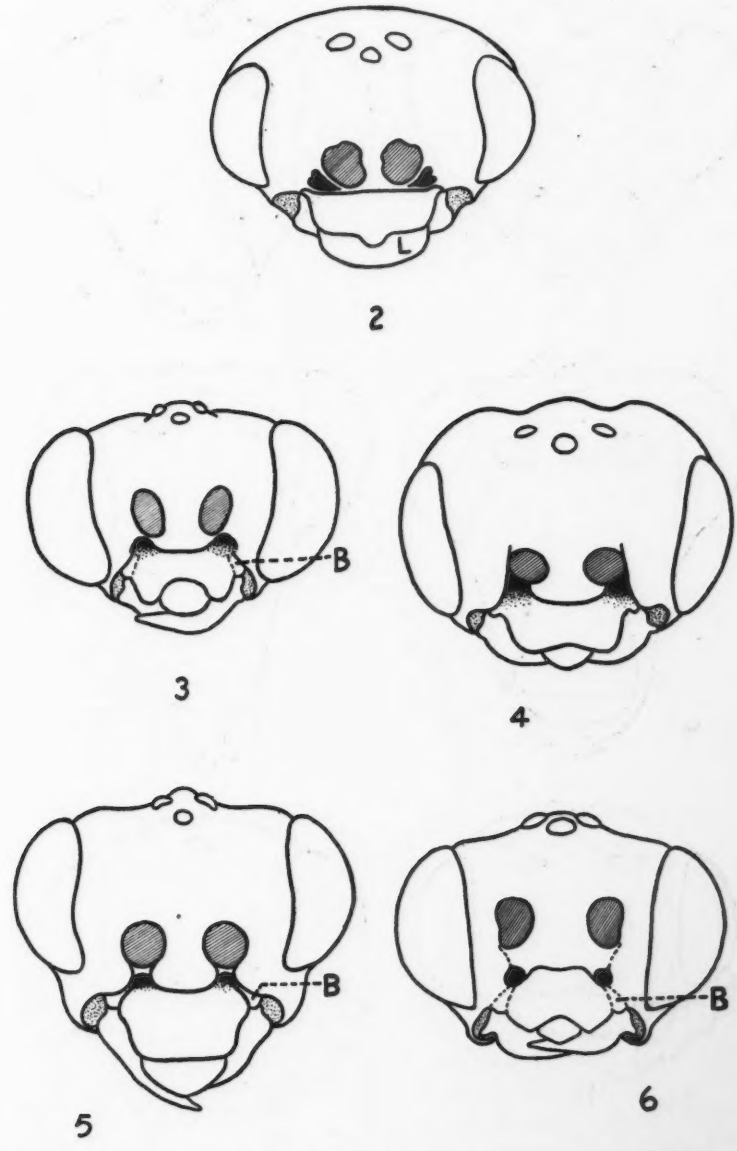


FIG. 2. *Pleroneura aldrichi* Ross, Xyelidae. FIG. 3. *Dimorphopteryx abnormis* Rohwer, male, Tenthredinidae. FIG. 4. *Blennogeneris spissipes* (Cresson), Tenthredinidae. FIG. 5. *Aglaostigma semiluteum* (Norton), male, Tenthredinidae. FIG. 6. *Ametastegia recens* (Say), male, Tenthredinidae.

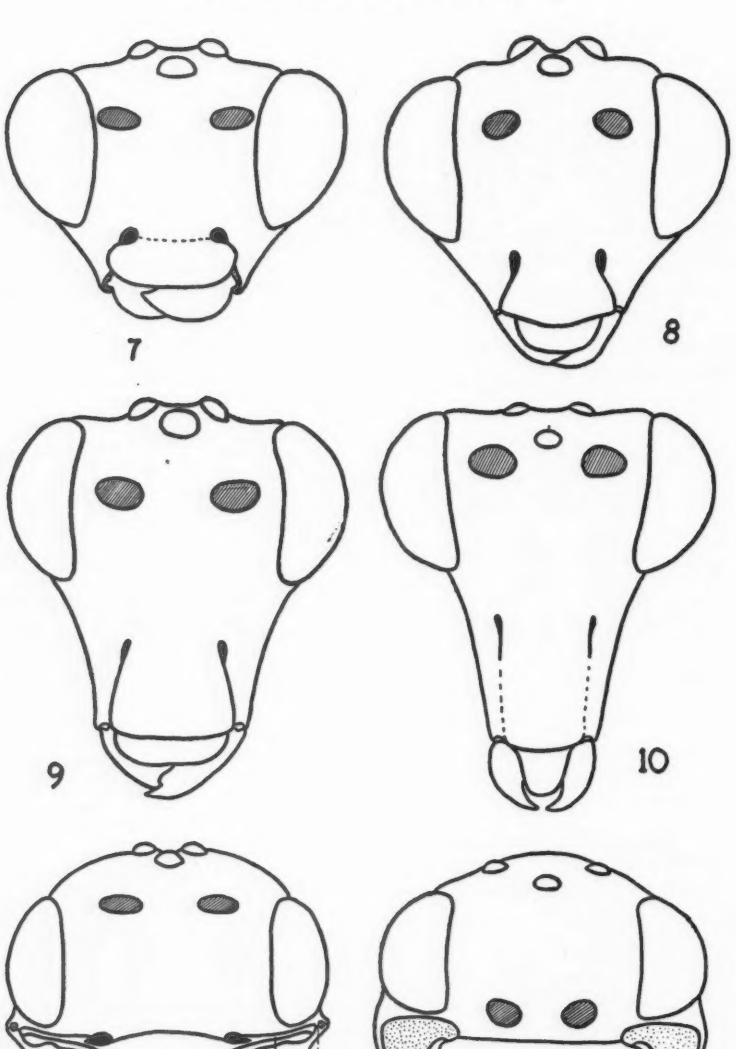


FIG. 7. *Agathis texanus* (Cresson), male, Braconidae. FIG. 8. *Agathis buttricki* (Viereck), female, Braconidae. FIG. 9. *Agathis atripes* Cresson, male, Braconidae. FIG. 10. *Cremnops vulgaris* (Cresson), male, Braconidae. FIG. 11. *Aphaereta* sp., female Braconidae. FIG. 12. *Vanhornia eucnemidarum* Crawford, Vanhorniidae.

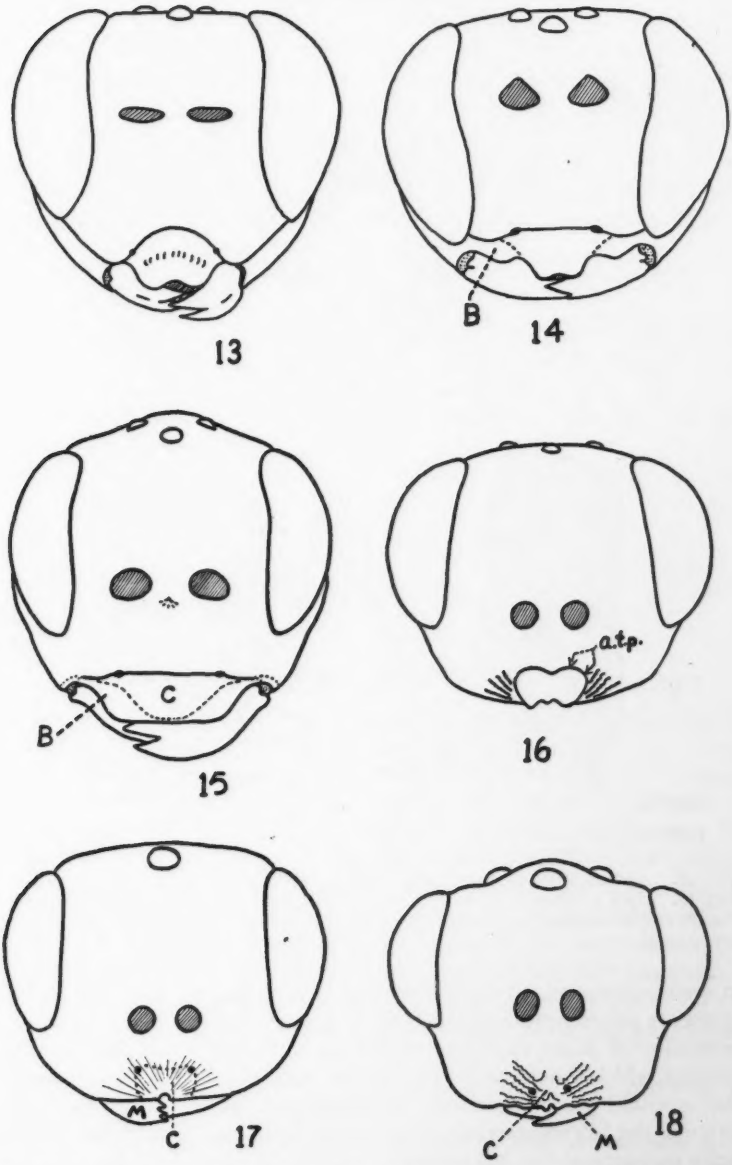
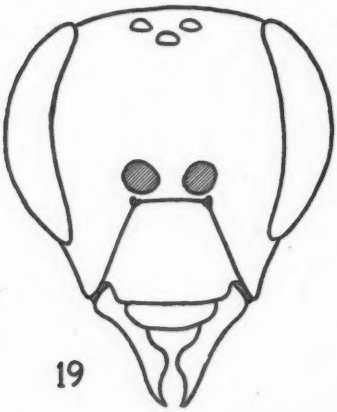
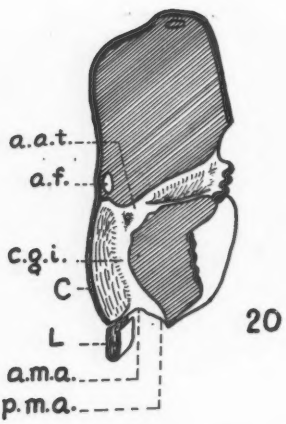


FIG. 13. *Coccygomimus aequalis* (Provancher), Ichneumonidae. FIG. 14. *Pseudorhyssa sternata* Merril, Ichneumonidae. FIG. 15. *Centeterus tuberculifrons* (Provancher), female, Ichneumonidae. FIG. 16. *Amblymerus verditer* (Norton), female, Pteromalidae. FIG. 17. *Psychophagus omnivorus* (Walker), female, Pteromalidae. FIG. 18. *Eurytoma pissodes* Girault, female, Eurytomidae. (The tentorial pits are not visible externally. The black spots indicate the points, determined by dissection, at which the tentorial arms meet the face.)





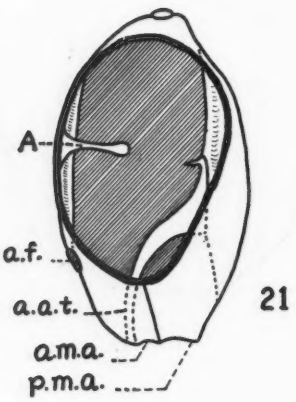


FIG. 19. *Apis mellifera* L., female, Apidae.
FIG. 20. *Apis mellifera* L., female, Apidae, internal view of head, lateral aspect.
FIG. 21. *Dahlbominus fuscipennis* (Zetterstedt), Eulophidae, internal view of head, lateral aspect.

No conclusions will be drawn here as to whether or not the similarities observed are convergent or parallel, as such conclusions must be based on the consideration of more than one character. Some of the more noticeable similarities, which should perhaps be studied more closely, will be pointed out.

The braconid genus *Ephedrus*, of the family Aphidiinae, resembles the majority of the Microgasterinae more closely in certain facial features than it does the majority of the Aphidiinae.

The mandibular orientation in the proctotrupoid *Vanhornia* (Fig. 12) is essentially like that in dacnusine braconids (Fig. 11). This is a very unusual modification in the Hymenoptera, and it would be interesting to compare the habits and structures of these two groups.

The facial type represented by *Coccygomimus aequalis* (Fig. 13) is common in the ichneumonid subfamilies Pimplinae and Diplazoninae, which are widely separated in Muesebeck *et al.* (4). It is also consistently present in the tribes Adelognathini, Phrudini, and Phytodietini, of the subfamily Tryphoninae, but not in other tryphonine tribes.

The rhyssine facial type is present in the genus *Rhyssa* of the ichneumonid tribe Rhyssini, in *Pseudorhyssa* (Fig. 14) of the Pimplini, and in *Clistopyga* and *Zatypota* of the Polysphinctini. In the last two tribes, not all genera show rhyssine facial characteristics.

Diacritus muliebris of the Pimplinae is more similar to many tryphonine species in facial type than it is to other pimpline species.

Chrysidoids, scolioids, sphecoids, formicids, gasteruptiids, and pelecinids share similar facial characteristics, in that the antennal foramina encroach upon the clypeal area in some species of all these groups.

Pteromalids resemble cynipoids much more closely than they do other chalcidoids in facial structure.

Similar surveys of different character complexes, including the entire order, and the subsequent comparison of results with those derived from facial structure, might provide useful information for the classification of the higher categories. It was impossible to include all the observations that were made on hymenopteran facial structure in this paper, but these could be made available for comparison.

Summary and Conclusions

The clypeus, in the Hymenoptera, is that region of the face between the clypeolabral, frontoclypeal, and clypeogenal inflections.

The anterior tentorial arms, and the related frontoclypeal and clypeogenal inflections, form a distinct supporting structure that provides rigidity to the anterior ventral region of the face between the mandibular articulations. The boundary between the clypeus and frons can be determined from the positions of the anterior tentorial pits. If no frontoclypeal inflection is present, this boundary may be taken as a straight line between the anterior tentorial pits.

Dorsoventral separation of the anterior tentorial pits and the anterior mandibular articulations has been accomplished in the Hymenoptera by ventral extensions of the genae as proposed by DuPorte and Bigelow (2) and not by dorsal migration of the epistomal suture and tentorial pits as proposed by Snodgrass (7).

Enclosure of the clypeus between the genae probably has preceded its reduction in many species of Hymenoptera.

Reduction of the clypeus has been accomplished by ventral extension of the frontal region above the level of the anterior tentorial pits and by mesal extensions of the genae in many species of Hymenoptera.

Certain sclerites and integumental grooves apparently can be created or obliterated relatively quickly and therefore must be used with considerable caution in the comparison of distantly related groups.

Similarities in facial structure exist between groups that are widely separated by taxonomists, as indicated by Muesebeck *et al.* (4), which must be due to either convergent evolution or unnatural arrangement in the catalogue. Surveys of other character complexes, when compared with the results obtained from the facial structure, might provide useful evidence for the natural classification of the higher categories.

Acknowledgments

This paper is a summary of research that was carried out in partial fulfillment of the requirements for the degree of Doctor of Philosophy at McGill University. This work was made possible by financial assistance from the National Research Council of Canada, which is gratefully acknowledged. Valuable assistance was received from the officers of the Systematic Entomology Unit, Science Service, Department of Agriculture, Ottawa, and particularly from Mr. G. S. Walley, Dr. O. Peck, Dr. R. Lambert, Dr. W. R. M. Mason, and Mr. C. D. F. Miller, who made the Hymenoptera in the Canadian National Collection available for study. Valuable assistance and advice was received from my director of research, Dr. E. M. DuPorte, Macdonald College.

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UPPER LETHAL TEMPERATURE RELATIONS OF THE GUPPY, *LEBISTES RETICULATUS*[1]

By M. Beatrice Gibson

Abstract

Guppies, *Lebistes reticulatus*, of inbred and unselected stocks, were tested for their variability in resistance to upper lethal temperatures. Unselected fish were maintained in constant temperatures of 20°, 25°, and 30° C. from birth and were subjected to lethal temperatures either without further treatment or acclimated to 30° C. before testing. Inbred lines were reared at 25° C. and acclimated to 30° C. Resistance times were determined at constant temperatures ranging from 33° to 38° C. Acclimation has a moderate influence on the resistance times at the higher lethal temperatures, but the effect is lessened at 35° C. and disappears at 34° C. There is an indication of an optimum rearing temperature in the neighborhood of 25° C. for resistance to the higher temperatures. General response to high lethal temperatures is similar to that reported for other fish, but heterogeneity is exhibited at 37° and 34° C. Both genetic composition and early thermal history influence resistance to high temperatures as well as acclimation just prior to experiment. The upper incipient lethal temperature is slightly above 32° C. over the biokinetic range of the guppy.

Introduction

Ordinarily it is possible to obtain consistent results in studies of the upper lethal temperatures of fish when precautions have been taken to stabilize the thermal history of the subjects for certain periods of time immediately prior to any particular experiment. This procedure has been termed "acclimation", Brett (1), Fry, Hart, and Walker (3), Hart (5, 6). With the guppy, application of the technique of thermal stabilization did not produce the uniformity in response usually to be found.

A tentative interpretation suggested that genetic differences together with variation in temperature during rearing might have been factors influencing the variability in response.

This paper presents the results of an attempt to explore these possibilities.

Materials and Methods

The guppy, *Lebistes reticulatus*, of the family Poeciliidae, is a native of the West Indies and northeast South America. Haskins (7) reports that although it is a typical inhabitant of fresh-water streams, it ranges down into the brackish lagoons where the streams enter the sea. It also swarms in the rain-water pools on the surface of the asphalt lake in Trinidad.

The fish used in these experiments were domestic stock obtained from several Toronto sources and their place of origin was unknown. The various lots were pooled and parents were taken from this stock as needed to provide broods born and raised under constant conditions. These fish from the general stock are termed "unselected" in the description of experiments.

[1] *Manuscript received June 21, 1954.*

Contribution from the Ontario Fisheries Research Laboratory, University of Toronto, and the Ontario Department of Lands and Forests, Toronto, Ontario.

In addition a gravid female guppy chosen at random was segregated in 1948 as the parent to begin inbred lines. From her progeny a male was mated successively to two female siblings. From these matings three groups have been bred by brother × sister matings in each generation. The lines have been designated "*a*", "*ad*", and "*b*".

Unselected gravid females were placed at one of three constant temperatures, 20°, 25°, and 30° C., and the resulting young were reared at the temperature in which they were born. These were the fish used in the experiments. Certain groups of the fish raised at 20° and 25° C. were placed directly into the lethal baths, others were acclimated to 30° C. before being subjected to lethal temperatures. All the inbred stocks which were born and raised in 25° C. were then acclimated to 30° C. before the experiments.

These temperatures, 20°, 25°, and 30° C., cover the range over which the guppies could be induced to give birth. Several attempts to have gravid females produce young at 15° C. resulted only in the death of the females. Difficulty was encountered at 20° C. but eventually two females bore young, and these progeny continued to breed among themselves at that temperature. Therefore, the 20°C. reared fish cannot be regarded as unselected stock in the same sense that the term is applied to fish reared at other temperatures.

The aquaria used for rearing were heated with electric heaters in pyrex tubing controlled by incubator wafer regulators enclosed in lucite. Later a small room was equipped with a thermostat and special steam line and was maintained at approximately 78° F. (25.5° C.). Water temperatures in this room ranged from 24° to 26° C. with an occasional wider range which was quickly corrected. After this room was in operation individual baths regulated at 25° C. were dispensed with. At 20° C. the temperature was maintained by introduction of a flow of cold water regulated by a solenoid valve.

For the lethal tests, groups of fish, usually 10 individuals, were taken from the given acclimation or rearing temperature, placed directly into the test temperature, and the time noted. Three-gallon battery jars with 50-watt heaters were used for the lethal baths. Temperatures were kept within ± 0.1° C. of the test temperature by means of the type of regulators and relays described by Brett (1). A steady flow of air through a porous diffuser kept the water in these jars circulating and also well aerated.

The results were plotted on probability × logarithmic paper using percentage mortality × log of time to death in minutes. In most cases lines were fitted by eye through the points, and the medians and the standard deviations were read from the graphs. In certain instances the geometric mean time to death was calculated. These cases are indicated in the text.

Results

The Relation of Time to Death to Lethal Temperature

Fig. 1 shows the relation of the logarithm of the time to death to the mortality suffered by each sample as expressed in probits, of fish reared at

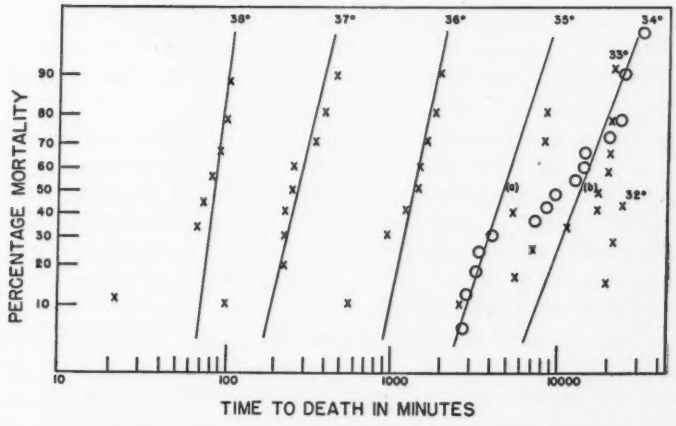

FIG. 1. Times to death at various lethal temperatures of guppies born and reared at 30° C. The circles show the results of the test at 34° C.

30° C. and exposed directly to various lethal temperatures. Each of the probit lines represents a single test except in the cases of 33° and 34° C., where the points are a composite of two tests.

For the samples at 38°, 37°, 36°, and 35° C. the points form reasonably straight lines although the point for the first fish to die was out of line with the others except at 35° C. It was assumed that these individuals had been weakened by handling. The slopes of the lines are essentially parallel and the lines are approximately equidistant. A decrease of 1° C. over the range from 38° to 35° C. increases the median resistance time about four times.

At 34° C. a different picture is presented. While the decrease of 1° C. from 36° to 35° C. increased resistance time over fourfold, the first 30% to die at 34° C. died as rapidly as did the corresponding fraction at 35° C. Moreover, at 34° C. the pattern of mortality does not fit a single probit line. One group of the sample forms a line coinciding with a section of that illustrating the mortality at 35° C., as is mentioned above. Another group forms a line much later in time. This phenomenon has been designated a "split probit" and was recorded in lethal temperature tests with Pacific salmon by Brett (1). In the graph these two phases have been given separate lines and from these, two theoretical medians, labelled (*a*) and (*b*), have been found by extrapolation. These medians are presumably those which would have resulted if one or other of the responses had been obtained singly.

Further, at 34° C. there is a wide range in individual times to death. At 38° to 35° C., the difference between the logarithms of times at the mortalities represented by four and six probits averages 8% of the logarithm of the median time in the tests. In contrast, at 34° C. a similar measurement of variability gives a value of approximately 20%. To make this latter estimate, the median was taken as 10,000 min., which is the general median of the heterogeneous data at this latter temperature.

At 33° C. there was again an overlapping of the resistance times with those of the lethal temperature 1° C. higher. Although the fish in 33° C. lived longer than those composing the "first phase" in 34° C. they all died within the range of the "second phase".

An attempt was made to test a sample of these 30° C. reared fish at 32° C., and the trend of deaths at this temperature is shown in the figure. Unfortunately, an accident prevented carrying through this test to completion so that only the early mortality could be followed. The times to death of the first fish to die overlap the second probit line of the 34° C. sample. Since it was possible to rear a few fish at 32° C. it is reasonable to believe that in this test some fish would have survived.

Fig. 1 gives a typical picture of the results within any one of the thermal histories studied. The response to the higher lethal temperatures is similar to that found by workers with other fish, Fry *et al.* (3), Brett (1), Hart (5, 6). That is, with each decrease in lethal temperature of 1° C. from 38° to 35° C. the guppies have a regular increase in resistance time. At 34° C., however, the various factors causing death seem to have different effects on individual fish. The factor or factors that kill the fish in the first phase do not affect in the same way those fish which continue to live on into the second phase. Apparently it is a different factor that kills the fish in the second phase.

Figs. 2*A* and *B* show the time relation to death of a group of fish also acclimated to 30° C. but reared at 25° C. The details of acclimation of this group are given on page 398. These graphs show essentially the same pattern as has been described for Fig. 1. With 25° C. reared fish acclimated to 30° C., the times to death at 35° and 34° C. overlap and both break into two phases (Fig. 2). Three separate tests were carried out at 34° C. and the fish in all three fell into the two phases, showing a very great spread in times to death among those in the second phase. Three tests at 35° C. gave two even more distinct phases. In Fig. 2*B* a composite plot of the three tests arranged in order of time to death is shown separately for both 35° and 34° C. to allow them to be seen clearly. Experiments with fish reared at 20° and acclimated to 30° C. resulted in a break at 34° C. only.

Samples of the unselected fish tested directly from their rearing temperatures of 20° and 25° C. showed essentially the same type of variability as described above for the groups reared at these temperatures and acclimated to 30° C. In the fish reared at 20° C. until tested the same wide distribution among individual times to death occurred at 34° C. as was obtained with the 30° C. reared fish. There was also a distinct break into two phases. Although the fish reared at 25° C. until tested did not give the same type of response at 34° C., a test at 33° C. with these fish did result in the split probit. The first two fish died at 7000 min. and the median of the next six to die was 40,000 min. Two fish survived for at least 60,000 min. when the experiment was discontinued.

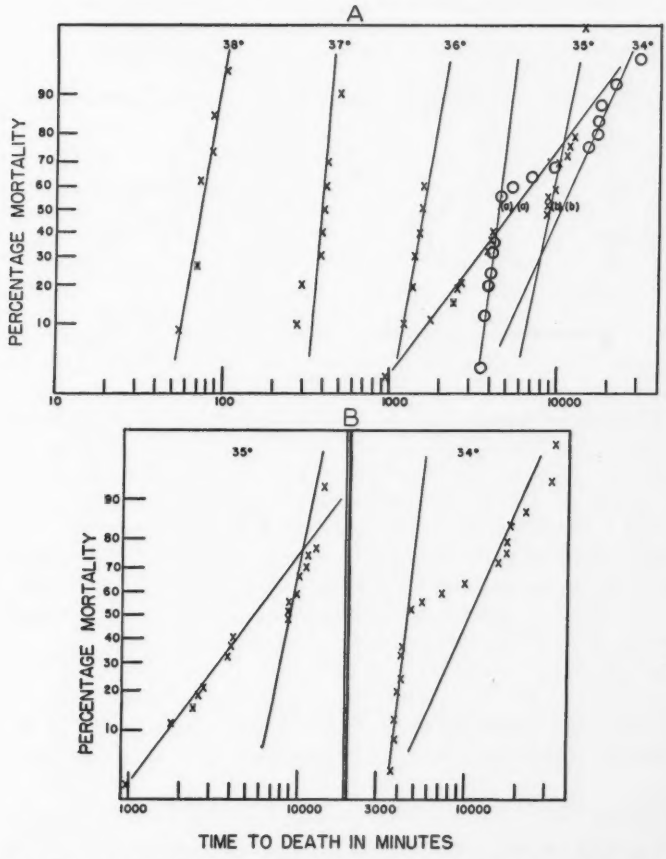

FIG. 2*A*. Times to death at various lethal temperatures of guppies born and reared at 25° C. and acclimated to 30° C. The circles show the results of the test at 34° C. FIG. 2*B*. Times to death of the fish shown in Fig. 2*A* at 35° and 34° C. Plotted separately to illustrate the wide distribution of deaths and the "split probits".

Effect of Thermal Acclimation on Lethal Temperatures

Fig. 3 illustrates the geometric mean times to death of samples of fish raised at 20°, 25°, and 30° C. and placed in the lethal baths directly from these temperatures.*

In the range 38° to 36° C., from the highest to the lowest rearing temperature the mean resistance times were progressively shorter in fish reared at lower

** It should be pointed out that certain of the points on the graph show the geometric mean time to death or more than the usual 10 fish. At these temperatures more than one experiment was carried out. For example, three different tests were done at 37° C. with the 25° C. reared guppies, comprising a total of 29 fish, because the results from the first trial appeared different from the usual regular increase of resistance time at 37° C. over that at 38° C. At 34° C. with 30° C. fish and at 37° C. with 20° C. fish also, the points are from a composite of two tests of 17 and 18 fish respectively. At 33° C. with 30° C. fish, 19 individuals were included.*

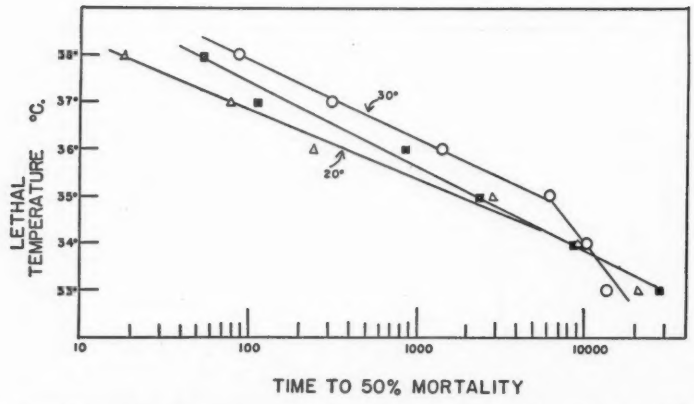

FIG. 3. Mean resistance times to lethal temperatures of guppies reared at 20°, 25°, and 30° C. Each point is the geometric mean time to death of the sample.

temperatures. At 35° C. there was a reversal of position of the means of the 20° and 25° C. reared fish from that at the higher lethal temperatures, while the position of the sample from 30° C. was maintained. The over-all difference in effect brought about by previous thermal history decreases also at 35° C. The relative spread of the means as measured by the logarithmic difference is 0.34 at this temperature whereas it averages 0.69 at the three higher temperatures. At 34° C., times to death were practically identical in all three groups. At 33° C., while the spread had again returned to a value of 0.33, there was a reversal in the order of sensitivity in that 25° C. reared fish were the hardiest and the 30° C. group were the most sensitive.

Effect of Early Thermal History on Response to Lethal Temperature

Three groups of fish are included in this comparison which were reared respectively at 20°, 25°, and 30° C. The 30° C. reared fish were placed directly into the lethal baths, those reared in 25° C. were kept in 30° C. for a period of 10 to 14 days before lethal experiments were carried out with them. The 20° C. fish were kept at 25° C. for periods of about 10 days and then at 30° C. for 10 to 14 days further before testing.

The results from the three highest lethal temperatures, 38°, 37°, and 36° C., were subjected to an analysis of variance under the guidance of Dr. D. B. DeLury, Ontario Research Foundation. For statistical balance the tables were set up with the times to death of eight fish in each case. These were chosen at random whenever there were more than eight to choose from except in the 30° C. reared group where the first fish to die at the three temperatures being studied were very much out of line with the others (Fig. 1). In this case these points were omitted, and the eight fish were chosen from the remainder. The first two fish to die at 37° C. in the 20° C. reared group were also discarded as being atypical.

TABLE I

SUMMARY OF ANALYSIS OF VARIANCE OF TIMES TO DEATH OF FISH REARED IN 20°, 25°, AND 30° C. AND ACCLIMATED TO 30° C.

	Degrees of freedom	Sums of squares	Mean squares	F	$F_{.01}$
Lethals	2	18.9283	9.4642	473	4.98
Rearing temps.	2	0.2683	0.1342	8.6	4.98
Lethals × rearing temps.	4	0.0794	0.0198	1.2	3.65
Within samples	63	0.9830	0.0156		

Table I presents a summary of the analysis the results of which demonstrated that: (1) there was no significant variation due to interaction between rearing temperatures and lethal temperatures, (2) the variance among the lethal temperatures was very large, which of course was expected, and (3) there were significant differences among the three rearing temperatures. There was an indication that the relationship of these differences was not a linear one, and Table II shows that the deviation from linearity is significant. Somewhere in the range from 20° to 30° C., and in the vicinity of 25° C., there was an optimum rearing temperature which enabled the fish to better resist high temperatures regardless of the final acclimation.

TABLE II

ANALYSIS TO SEPARATE OUT THE LINEAR AND QUADRATIC COMPONENTS OF THE CURVE RELATING LOG TIME TO DEATH WITH REARING TEMPERATURE

				Sums of squares
Rearing temps., ° C.	20.0	25.0	30.0	
Sums	58.22	61.70	60.72	
Linear component	− 1	0	1	0.1302
Quadratic component	− 1	2	− 1	0.1381

Both linear and quadratic components are significant. Hence the curve relating log time to death with rearing temperature rises to a maximum and then declines.

Fig. 4 illustrates the resistance lines of the fish from these three rearing temperatures acclimated to 30° C. The points for the three highest lethals are the means of the times to death of the eight fish used for the analysis of variance in each case. The points at 35° and 34° C. are the geometric mean times to death of all the tests at these temperatures, as illustrated in Figs. 1 and 2. The trend of the times to death at the three highest lethal temperatures is continued down to 35° C., and it would have been logical perhaps to include this temperature as a fourth category in the analysis of variance.

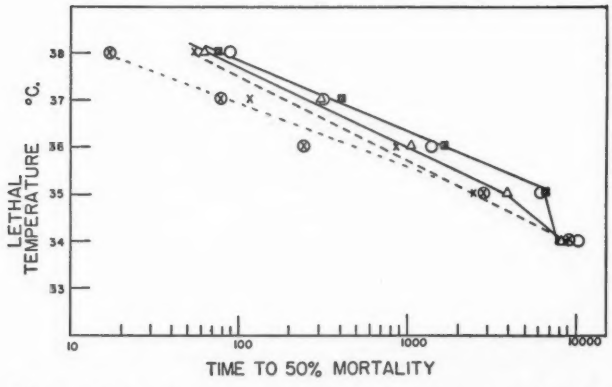

FIG. 4. Mean resistance times to lethal temperatures of guppies of various thermal histories. Circles show the 30° C. reared fish. Squares show the 25° C. reared fish, acclimated to 30° C. Triangles show the 20° C. reared fish, acclimated to 30° C. Crosses show the 25° C. reared fish. Circled crosses show the 20° C. reared fish.

However, the variance of the 25° C. reared sample (Fig. 2*B*) was so much greater than that of the other groups that it was felt that the disturbance in relations found in all cases at 34° C. might be already entering in at 35° C. In any event inclusion of the 35° C. points would not make any change in the conclusions drawn from the data for the higher temperatures. The pattern displayed down to 35° C. is not maintained at 34° C., as is pointed out on page 396. At this latter temperature death came much sooner than would have been predicted from the events at the higher levels.

The dotted lines included in Fig. 4 show the data for the fish maintained until testing at 20° and 25° C. At lethal temperatures down to 35° C. it would appear that acclimation to 30° C. has increased the thermal resistance of the 20° C. reared fish to a slightly higher degree than that of fish whose entire thermal experience was at 25° C. At 34° C. neither acclimation temperature nor rearing temperature appears to have any influence on the resistance time. Since a complete series of tests at 33° C. was not carried out, the relation at this temperature is not known.

Variability Among Homogeneous Groups

Table III lists the median times to death and standard deviations taken from probit graphs of the various lethal tests done with the inbred fish and with crosses of two of the lines.

With the exception of the 38° C. test the "*a*" line showed lower resistance than unselected fish of the same acclimation and rearing, especially at 35° C. At 37° C., however, there was one male which survived over 1000 min. in contrast to the usual times which ranged from about 200 to 500 min. for both inbred and unselected fish. The "*a*" line does not display the ability of a certain fraction of the sample to withstand 34° C. for very long periods as described for the unselected fish.

TABLE III

MEDIAN TIMES TO DEATH AND STANDARD DEVIATIONS (IN MINUTES) OF INBRED LINES OF GUPPIES IN LETHAL TEMPERATURES

Inbred lines	Lethal temperatures in ° C.				
	38.0	37.0	36.0	35.0	34.0
"*a*" F_6	72 ± 8	185 ± 27	940 + 140 − 120		4400 ± 600
F_7	71 ± 7	330 + 90 − 70	1410 + 410 − 310	2920 ± 480	5200 + 700 − 640
F_8			880 + 220 − 180	2000 + 700 − 540	
"*ad*" F_7	63 ± 5		1720 + 220 − 200	1850 + 250 − 210	3900 ± 100
F_8		188 + 22 − 20			4700 ± 400
"*b*" F_6	61 ± 3	300 + 130 − 95	535 + 110 − 95	*a*. 1110 + 1170 − 120	*a*. 4250 + 950 − 750
				b. 9300 + 1900 − 1600	*b*. 10000 + 3600 − 2600
$F_6a \times F_6b$		*a*. 480 + 100 − 75	770 + 280 − 210		4200 + 750 − 650
		b. 1420 + 210 − 200			
$F_6b \times F_6a$		125 + 40 − 29	2550 + 1450 − 950	3290 + 1410 − 990	*a*. 5500 + 680 − 650
					b. 14400 + 3500 − 3000

In a test with the third generation this "*a*" line did show a prolonged resistance time at 34° C. when it seemed to be an attribute of the males which lived about 5700 min. longer than the females in this temperature. In tests with fifth, sixth, and seventh generations, however, although the females were first to die, the males did not have the same length of resistance times as they did in the third generation. This may indicate that the quality which enables some guppies to withstand the lethal factor (or factors) operating at 34° C. was already bred out of "*a*" line by the fourth generation.

The results from the "*ad*" line did not differ appreciably from those of "*a*". This "*ad*" line was from another brother × sister mating of "*a*" line in the fourth generation and kept separate after that. This perhaps reinforces the conclusion reached in the paragraph above.

On the other hand the "*b*" line does show the overlapping and split probits characteristic of the unselected stocks at 35° and 34° C. The range of survival times is not so great as with the unselected, but this may be because fewer fish were used in the lethal tests.

Reciprocal crosses were made with these two lines and tests of the progeny in lethal temperatures gave some rather curious results. Five females, progeny of $F_4a \times F_4b$, had a much longer resistance time at 37° C. than was usual with either inbred or unselected fish of this acclimation. Two other fish, a male and a female, died within the expected time. Theoretical medians for the complete test, obtained as described for unselected fish, are listed in Table III. Even the calculated geometric mean time to death of 1066 min. for all seven fish is twice as great as any other comparable group at this temperature. The opposite situation resulted from tests at 37° C. with $F_4b \times F_5a$. These fish were much more sensitive to this temperature than others appeared to be. As a check on the first experiment with nine fish a second was done three months later with seven fish of the same cross. These lasted longer than the first group tested, but were still much less resistant than the unselected fish and majority of inbreds. The nearest comparable times to death were obtained in an experiment with six males of "*a*" line, sixth generation.

There was a difference in response between the progeny of the two crosses at 34° C. also. With the $a \times b$ sample the times to death formed one probit line corresponding to the earlier line of the unselected fish. On the other hand the fish from $b \times a$ showed the two phases ordinarily found.

Variability Between the Sexes in Response to High Temperatures

In Fig. 5*A* times to death for all the inbred fish from three lines have been plotted with different symbols for the two sexes. At 35° and 34° C. the times to death mingled so closely that it was difficult to separate them by examining the graph. Therefore, the results at 34° C. have been plotted separately for clarity. From Fig. 5*A* it is apparent that there is a definite difference between the sexes in time of resistance to lethal temperatures at two points, 37° and 34° C., one of which is the reverse of the other. Mean times to death at 37° C. were 234 min. for males and 349 min. for females, while at 34° C. on the other hand the mean times to death were 3400 for females and 5660 min. for males, a distinct reversal of position. A comparison of the two sexes at 37° C. by means of the "*t*" test gave $P = .04$. The separation into two phases made it impossible to do a similar comparison for the 34° C. fish.

An indication of this sex difference was observed also in the tests at 34° C. with progeny of reciprocal crosses of two lines, particularly with the $b \times a$ cross. At 37° C., however, the fish from cross $b \times a$ (the group very sensitive to this temperature) showed no regular relationship between sex and time to death. The test at 37° C. with $a \times b$ fish was with six females, five of which as related above showed unusually long resistance times, and only one male which succumbed first of the group.

As Fig. 5*B* shows, the relationship is slightly different with the unselected fish. To make this graph the data on the fish from the three rearing temperatures and acclimated to 30° C. were combined. With these fish the lower resistance of the males at 37° C. was very marked and at 36° C. there was a

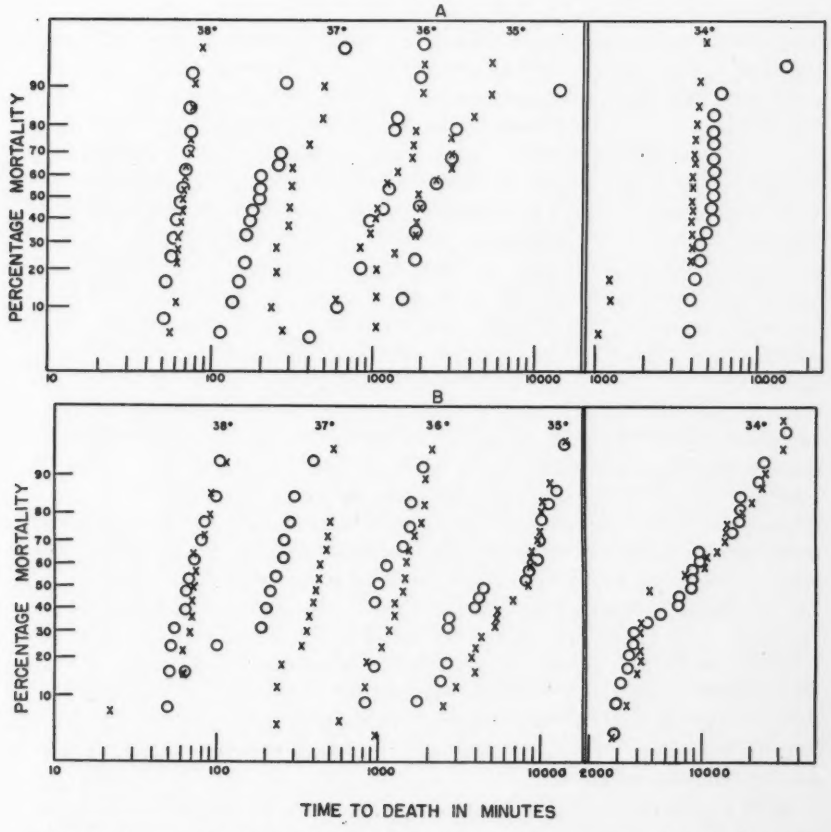

FIG. 5. Comparison of times to death in various lethal temperatures of males (circles) and females (crosses).
A—inbred lines. *B*—unselected stocks.
The samples at 34° C. are plotted separately for clarity.

slight indication of it. At 35° C. the males seem to have been less resistant in the first phase only. The over-all mean time to death of the females at this temperature was over 900 min. greater than that of the males. At 34° C. no difference between the sexes in response to temperature is apparent in these unselected fish.

Discussion

The foregoing results seem to show that the responses of the guppy to lethal temperatures are different from those usually encountered in fish, but the differences are of degree rather than kind. The most marked characteristic of its behavior is the absence of any change in its incipient lethal

temperature with differences in thermal history. The incipient lethal temperature has been defined by Fry *et al.* (3) as that level which on indefinite exposure just fails to kill 50% of the sample. This index was not determined precisely in the present experiments because it is necessary to subject these fish to temperatures at the lower end of their lethal range for such an extremely long time before they take effect. It is probably slightly above 32° C. Guppies acclimated to 30° C. will die at 33° C. On the other hand, guppies can be reared to maturity at 32° C., although with difficulty, and those reared by the present author did not reproduce.

Typically, over most of their biokinetic range fish will show an increase of about 1° C. in their upper incipient lethal temperature for about 3° C. change in their acclimation temperature, e.g. Fry, Brett, and Clawson (2). It is general, and indeed universal so far as data are available, Fry *et al.* (3), Brett (1), Hart (5, 6), for this increase in lethal temperature to be halted at the highest levels of acclimation temperature. For instance, in the case of the goldfish used by Fry, Brett, and Clawson (2) the incipient lethal level reaches the highest value it attains (just over 40° C.) at an acclimation temperature of 38° C. and remains constant from then on. The plateau displayed by the goldfish in this respect is very minor, but other fish have more extensive ones in their thermal tolerance diagrams. In young spring salmon (*Oncorhynchus tschawytscha*) the incipient lethal reaches approximately 25° C. at an acclimation temperature of 15° C. and does not change thereafter, Brett (1). The plateau of the upper lethal in the diagram therefore extends from 15° to 25° C. A similar extensive plateau was found for the minnow, *Rhinichthys atratulus*, by Hart (6) over the acclimation range of about 13° to 28° C. The thermal tolerance diagram for the guppy would show a horizontal line for the upper incipient lethal over the whole biokinetic range as far as our experiments indicate.

With regard to thermal resistance, Fry *et al.* (3), the guppy also represents an extreme. In the lower range of lethals near the incipient lethal level, experiments with the guppy have to be carried on very much longer than 10,000 min. before the mortality pattern is complete. The only comparable results found in the literature are those of Brett (1) for the chum and spring salmon.

In its pattern of change in thermal resistance time with increasing acclimation temperature, the guppy again tends towards an extreme. The differences in thermal resistance induced by a 10° C. increase in acclimation, i.e. from 20° to 30° C., are not very great when compared with other species of fish. For example, Brett's (1) data for young Pacific salmon show as much as a 13-fold increase in resistance time at the lethal temperature of 26° C. for an increase in acclimation temperature from 10° to 20° C. The smallest increase he found at these temperatures was a fivefold one in the coho (*O. kisutch*). With the guppy the greatest increase in resistance with 10° C. change in acclimation temperature was about sixfold. This occurred at a lethal temperature of 36° C.

The most noteworthy feature of the thermal resistance of the guppy was the entire lack of an acclimation effect at 34° C. Such performance has not been found in any other species to date, although a tendency for the resistance lines to converge at the lower temperatures of the lethal zone is seen in Hart's (6) data for *Gambusia affinis* and *Micropterus salmoides* at the higher acclimations.

Apparently the effects of thermal acclimation on the response of the guppy to lethal temperatures are rather slight as compared to those ordinarily found in fish. However, the problem under consideration here was whether there remained a residual variation after the acclimation effect had presumably been controlled. Such variation might be the effect of genetic variability or the consequences of irreversible changes brought about by early thermal history.

In the case of the guppy at the higher lethal temperatures, early thermal history has an effect which may be equivalent to about one third of the acclimation effect (see Fig. 4). This discovery may have a bearing on some of the anomalies Hart (6) found in the lethal temperatures of certain species when he compared samples from one locality with those of another. In particular, his data for *Gambusia affinis* illustrate behavior similar to that of the guppy brought about by temperature differences during rearing. It will be of interest to explore the effect of the early thermal history on other species more fully adapted to fresh water than the cyprinodonts are.

Further experimentation is also needed with the guppy, since, while the significant decrease in the resistance of the group reared at 20° C. seems to indicate susceptibility to high temperatures induced by the cold environment in which they were born and raised, it will be remembered that these fish were all from a population which originated from the only two females that could be induced to give birth at 20° C., and therefore cannot strictly be termed "unselected" fish.

The indication of an optimum rearing temperature with respect to temperature resistance, which is supported by the analysis in Table II, is borne out also by observations on the rate of growth. Fish reared at 30° C. never attained the size nor displayed the activity of those reared at either 25° or 20° C., Gibson and Hirst (4). The general mortality rate was also higher at 30° C. but no record was kept of the percentage survival. Shoemaker's (8) findings also show that general mortality sharply increased during periods when his aquarium temperatures went over 30° C. Thirty degrees centigrade is not the absolute ceiling for growth and development, however, for of one family of nine guppies born June 22, 1952, at 32° C. and maintained at that temperature, six were still living and were reaching maturity on October 10th. All these fish had developed very crooked backbones by July 18th although otherwise they appeared healthy. Five of these fish matured but no young were obtained from them.

With regard to the response of the guppy to lethal temperatures when effects due to rearing and acclimation have been removed, there appear to date to be two critical temperatures at which irregularities are observed in the response of the fish. These are 37° and 34°–35° C.

At 37° C. and below, death seems to have resulted from a variety of causes impossible to sort out at this time. At 37° C. there is a marked difference among the 30° C. acclimated fish in the response of the sexes. Males succumbed first in both unselected and inbred stocks. At lower temperatures this particular response is lost again, although at 34° C. a difference in the opposite direction is shown between the sexes in the inbred lines. The factor bringing about death in the neighborhood of 37° C. appears to be different from those factors which operate above and below this temperature.

The other obvious point at which additional or different factors seem to enter into the lethal effect is at about 35° C. At 35° C. the effect of thermal history begins to be reduced (see Fig. 4), and at 34° C. to be lost altogether as the same figure indicates. All groups of all thermal histories die in about the same length of time at that temperature. Moreover, there is a reduction in the extent to which lowering the temperature level by 1° C. increases the length of time the sample lives. This is shown by the overlapping of the probit lines in Figs. 1 and 2.

The irregularity of the probit lines obtained at 35° and 34° C. appears to indicate at least two factors causing death, and that certain individuals may be resistant to the first factor. In the "*a*" inbred line the ability to make the first adjustment was apparently lost between the third and fifth generations, since a test at the earlier generation showed this ability in the males, although these fish did not display as prolonged a resistance as was found in "*b*" line and the unselected fish. As only one or two pairs were chosen from each generation to carry on the lines, it is conceivable that the resistance was lost in the fish not used for breeding. The "*b*" line on the other hand retained the resistance at least to the fifth generation.

In addition to the evidence provided by the inbred lines for the genetic basis of the variation to be found at 34° C., a level where the environmental temperature had little influence, the cross breedings show an inherited effect at both 37° and 34° C. At 37° C. the environmental influence on the guppy is as great as was found for any temperature. The genetic effect here is measured as the difference between the logarithm of the geometric mean times to death of the two samples tested, and is about the same as the environmental effect on the unselected stocks when similarly measured, as follows:

Cross	Log mean time to death	Difference	Thermal history unselected	Log mean time to death	Difference
$a \times b$	3.0294		25° C. reared 30° C. acclim.	2.6096	
$b \times a$	2.0969	0.8738	20° C. reared	1.8921	0.7175

The ratio is 1.2 : 1.

At 34° C. the fish from one of the crosses, $b \times a$, displayed the ability to resist the first lethal factor (or factors) operating at this temperature and the other, $a \times b$, did not.

Acknowledgments

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THE INFLUENCE OF CARBON DIOXIDE ON THE UTILIZATION OF OXYGEN BY SOME FRESH-WATER FISH[1]

By Edgar C. Black[2], F. E. J. Fry[3], and Virginia S. Black[4]

Abstract

The influence of carbon dioxide on the utilization of oxygen by 16 species of fresh-water fish from Algonquin Park, Ontario, Canada, has been measured by sealing fish individually in bottles containing water adequate in oxygen and with various concentrations of carbon dioxide. At death the ambient respired water was analyzed for free carbon dioxide and oxygen. Results give specific curves, which show that the oxygen in the respired water at the time of death was higher when the tension of carbon dioxide was increased. When the tension of carbon dioxide was low, the oxygen left in the water ranged from a tension of 4 mm. Hg for the northern brown bullhead, *Ameiurus nebulosus*, to 19 mm. Hg for the common brook trout, *Salvelinus fontinalis*. Carbon dioxide tensions causing death when a tension of 160 mm. Hg of oxygen remained in the respired water ranged from 80 mm. Hg for the northern blacknose shiner, *Notropis heterolepis heterolepis*, to 338 mm. Hg for the northern brown bullhead.

Introduction

The various environmental conditions which affect the respiration and metabolism of fishes were formulated by Krogh and Leitch (15) who investigated the capacity of blood of fishes to transport oxygen. Their results indicated that blood of certain fishes living in oxygen–poor environments is adapted to a low tension of oxygen. Further, their results showed that carbon dioxide exerted a greater effect on unloading oxygen from the blood than is the case for mammalian blood. Krogh and Leitch considered this enlarged Bohr effect as an adaptation of fish blood to low temperatures. The increased tension of oxygen discharged from the blood at the site of respiring tissues would offset in part the tendency of the blood to retain oxygen at low temperatures. Root (19) showed in a series of marine fish from the temperate zone that a very much larger Bohr effect operated than was formerly believed for fishes. For some of the species which Root studied, a tension of 15 mm. of carbon dioxide prevented the saturation of blood by oxygen even up to a tension of 500 mm. Willmer (24) found a wide variation in the Bohr effect of the bloods of some tropical fresh-water fishes. Black (1, 3) demonstrated, for a series of four fresh-water fish, differences in the affinity of fish blood (hemoglobin) for oxygen and also differences in the ability of carbon dioxide

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to reduce the oxygen-binding effect on blood. These two sets of differences were specific in nature. Furthermore it appeared that fishes with a high affinity for oxygen and a low Bohr effect inhabit the warmer bays of water, whereas a fish with lowered oxygen affinity and an increased sensitivity to carbon dioxide inhabits the deeper colder waters.

Carbon dioxide assists the discharge of oxygen at the tissues, but conversely prevents the loading of oxygen. It would seem, then, that the whole organism should show differences in its ability to load the blood at the gills according to the oxygen-carrying characteristics of the blood (3). Redfield and Goodkind (18) tested the ability of the squid to oxygenate its blood when an external respiratory medium was charged with various tensions of carbon dioxide. They observed that carbon dioxide interfered with oxygenation. Wiebe, McGavock, Fuller, and Markus (23) and also Powers and his associates (16) showed that fishes differ in their ability to utilize oxygen at various concentrations of acid. The prevention of utilization of oxygen by addition of carbon dioxide to the external milieu was reported for a series of fresh-water fishes by Fry and Black (6) and Fry (4). The present work is a continuation of these studies wherein the variability of the results was reduced by testing the response at the temperature of acclimatization. These studies were interrupted in 1940. The data are recorded here in the hope that they may be of interest to future workers.

In this paper, use of the term acclimation is restricted to the exposure of an organism to temperature conditions in the laboratory, whereas the term acclimatization refers to temperature changes that are imposed by the natural habitat.

Material and Methods

Sixteen species of fresh-water fishes were investigated at the summer laboratory of the Ontario Fisheries Research Laboratory, Opeongo Lake, Algonquin Park, Ontario, Canada, during the summer months of 1938, 1939, and 1940. Detailed information as to the common and scientific names of species, numbers used, average weights and weight ranges, temperatures at which experiments were carried out, tensions of oxygen remaining after lethal tests when no additional carbon dioxide was added, and the lethal tensions of carbon dioxide at a tension of oxygen of 160 mm. Hg, are listed in Table I. These species represent about a half of those available in Algonquin Park. The arrangement used in Table I follows the taxonomic sequence given by Hubbs and Lagler (11). The nomenclature for the burbot is taken from a recent paper by Speirs (22).

The fish were captured in a variety of ways from lakes and streams in the vicinity of the laboratory on Opeongo Lake in Algonquin Park. They were retained for at least 24 hr. after capture before being used in the experiments. Where the temperature at the site of capture was different from the temperature at which the fish were retained, sufficient time elapsed to allow acclimation to the temperature of captivity.

TABLE I

COMMON AND SCIENTIFIC NAMES, NUMBERS USED, AVERAGE WEIGHTS AND RANGES, EXPERIMENTAL TEMPERATURES, OXYGEN TENSIONS AT LOW CARBON DIOXIDE TENSIONS, AND LETHAL LIMITS OF CARBON DIOXIDE WHEN THE OXYGEN TENSION IS FIXED AT 160 MM. Hg FOR SPECIES STUDIED AT ALGONQUIN PARK, ONTARIO, JULY–AUGUST, 1940

Species	No. of fish used in experiment	Average and range of weight, in gm.	Average and range of temperature of experiment, in ° C.	P_{O_2} as mm. Hg at low CO_2	P_{CO_2} as mm. Hg at 160 mm. P_{O_2}
Common brook trout *Salvelinus fontinalis fontinalis* (Mitchill)	37	17.5 6.5–44	18.7 17–20	19	132
Common white sucker *Catostomus commersonnii commersonnii* (Lacepède)	28	265 153–454	17.1 15–18.5	9	107
Northern creek chub *Semotilus atromaculatus atromaculatus* (Mitchill)	32	13.9 4.0–26.0	18.8 17–21	8	234
Northern pearl dace *Margariscus margarita nachtriebi* (Cox)	25	5.3 3.0–11.0	18.6 18–19	5	186
Finescale dace *Pfrille neogaea* (Cope)	38	4.2 2.5–9.5	19.7 18–21	6	202
Northern redbelly dace *Chrosomus eos* Cope	36	2.3 1.5–3.5	20.0 19.8–20.0	14	182
Northern common shiner *Notropis cornutus frontalis* (Agassiz)	25	27.5 5–52	19.1 17.2–21.5	12	102
Northern blacknose shiner *Notropis heterolepis heterolepis* Eigenmann and Eigenmann	28	2.2 1.0–3.0	19.2 19–20	12	80
Brassy minnow *Hybognathus hankinsoni* Hubbs	12	4.0 3.0–6.0	19.4 18–20	7	96
Northern fathead minnow *Pimephales promelas promelas* Rafinesque	50	3.9 2.0–6.0	20.4 18–21	6	122
Northern brown bullhead *Ameiurus nebulosus nebulosus* (LeSueur)	30	36.1 15–65	20.1 19–22	4	338
Yellow perch *Perca flavescens* (Mitchill)	32	78 11–210	20.2 19–24	7	112
Northern smallmouth bass *Micropterus dolomieu dolomieu* Lacepède	23	255 26–1080	19.2 15–25	12	97
Pumpkinseed *Lepomis microlophus* (Gunther)	32	24.2 2–95	20.1 19.5–21.1	6	122
Brook stickleback *Eucalia inconstans* (Kirtland)	34	0.60 0.4–0.8	20.6 20.0–22.7	14	193
Eastern burbot *Lota lota lacustris* (LeSueur)	21	830 240–1540	15.4 12–18	13	112

Individual fish were placed in bottles of water containing adequate quantities of oxygen, that is, 150 mm. Hg tension or above, and at various tensions of carbon dioxide. The mixtures of dissolved carbon dioxide and oxygen were prepared by adding water charged with gaseous carbon dioxide and, where necessary, with additional water saturated with gaseous oxygen. Preliminary analyses for both carbon dioxide and oxygen were made for every experiment.

The size of the bottle used corresponded to the size of the fish. In general the quantity of oxygen present initially was such that the animal respired for at least an hour. The experimental bottles were sealed and placed in a water bath. When all respiratory movements had ceased the bottles were opened and two samples of water were carefully siphoned into appropriate containers for analysis of dissolved carbon dioxide and oxygen.

If a quantity of oxygen less than 10% of the initial value had been utilized by the fish during the asphyxiation experiment, the datum was not used. This level of 10% is an arbitrary one.

Free carbon dioxide was analyzed by titrating a 50-ml. sample of water with $M/44$ sodium hydroxide, using phenolphthalein to indicate the end point. The titration method was checked by determining the carbon dioxide found in the gas phase following equilibration of 0.5 ml. of air with a 35-ml. sample of water (21). A straight-line relationship was found between 0 and 280 mm. Hg (0–640 mgm. carbon dioxide per liter) (Fig. 1).

Dissolved oxygen was determined by the unmodified Winkler method. While there is no doubt that occasional organic material in the sample may have interfered with the iodimetric titration, no interference was noted in the check analyses made from time to time. The tension of oxygen was calculated by relating the quantity of oxygen found to the solubility of oxygen in water at the temperature of the experiments and multiplying the fraction by 760.

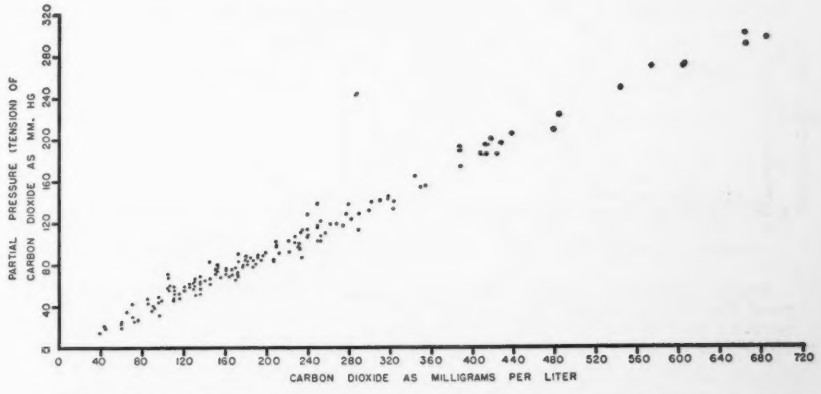

FIG. 1. Carbon dioxide tension as mm. Hg (Krogh method) plotted against carbon dioxide as milligrams per liter (titration method) at 20° C. Opeongo Lake water.

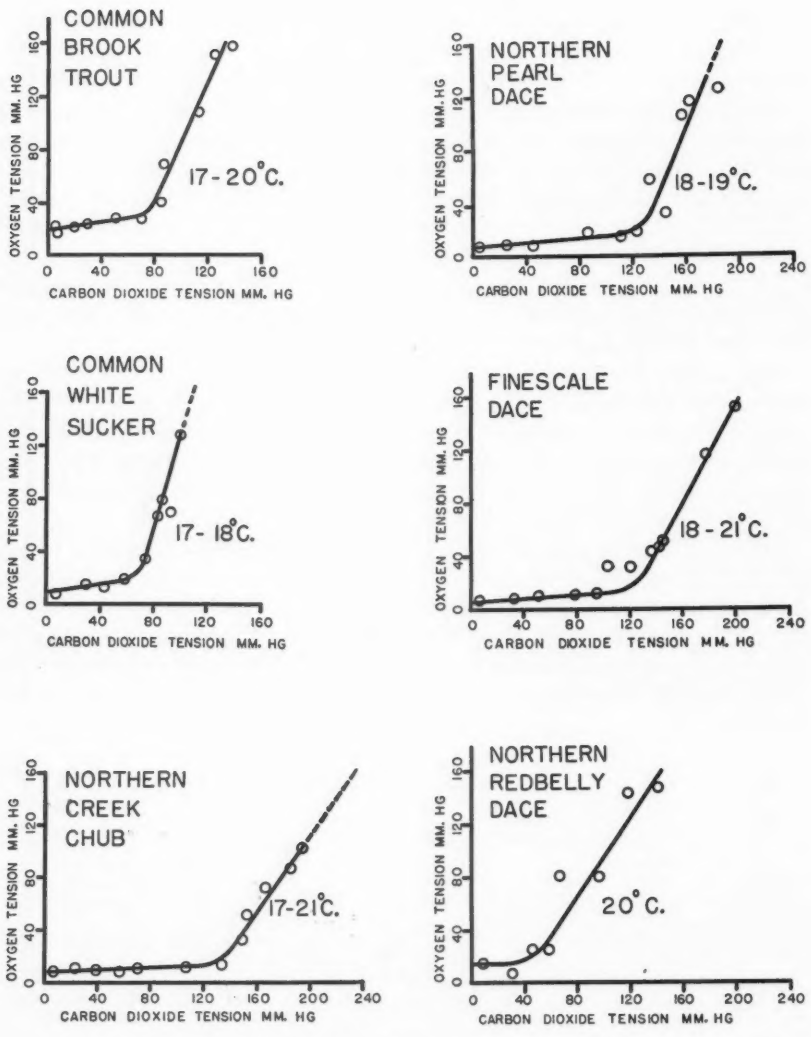

FIG. 2. Influence of carbon dioxide on the utilization of oxygen by the common brook trout (18.7° C.), common white sucker (17.1° C.), northern creek chub (18.8° C.), northern pearl dace (18.6° C.), finescale dace (19.7° C.), and northern redbelly dace (20.0° C.).

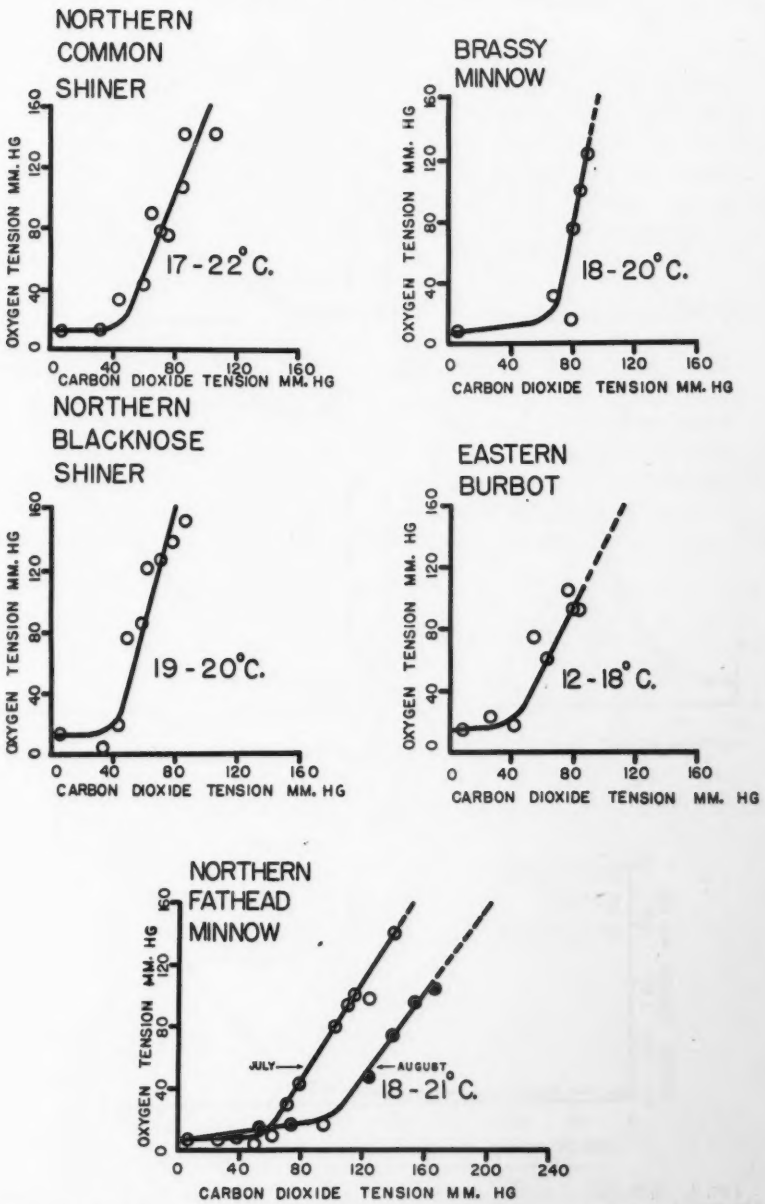

FIG. 3. Influence of carbon dioxide on the utilization of oxygen by the northern common shiner (19.1° C.), northern blacknose shiner (19.2° C.), brassy minnow (19.4° C.), eastern burbot (15.4° C.), and northern fathead minnow for July (20.4° C.) and August (19.7° C.).

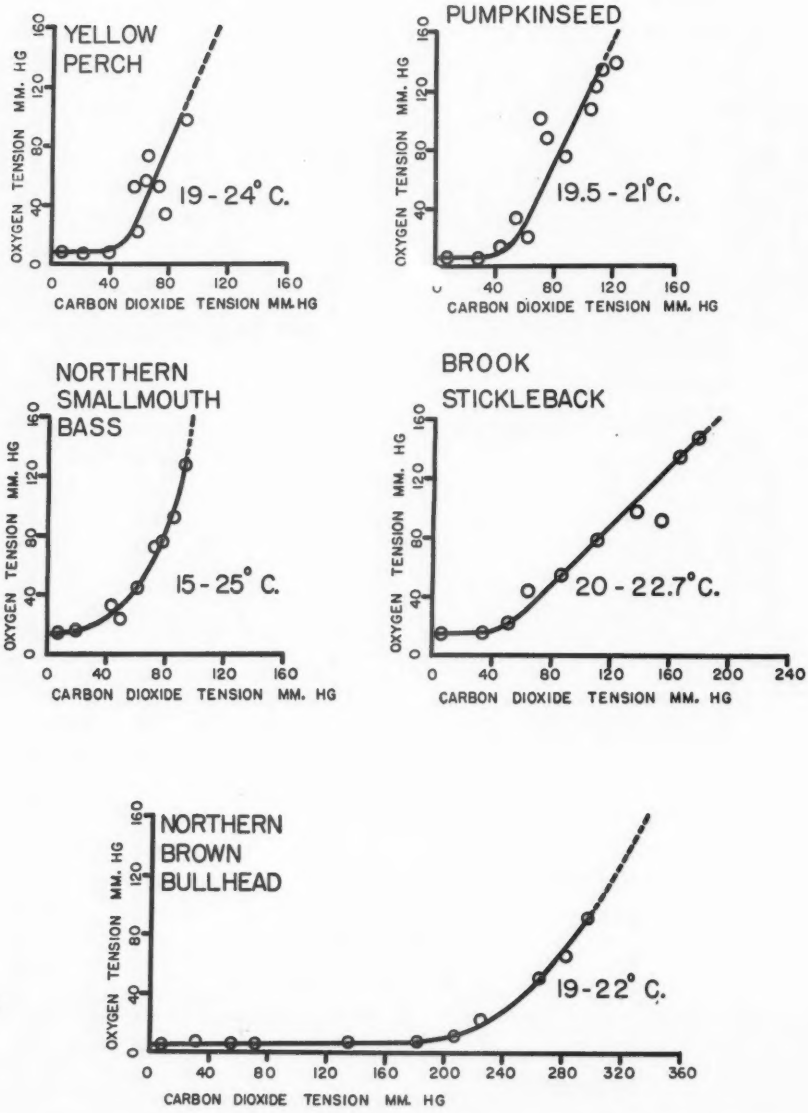

FIG. 4. Influence of carbon dioxide on the utilization of oxygen by the yellow perch (20.2° C.), northern smallmouth bass (19.2° C.), pumpkinseed (20.1° C.), brook stickleback (20.6° C.), and northern brown bullhead (20.1° C.).

Results

The tension of oxygen at death when no carbon dioxide was added to the water ranged from values as low as 4 mm. Hg for the northern brown bullhead through a value of 9 mm. Hg for the common white sucker to a value of 19 mm. Hg for the common brook trout. These values are of the same order as the oxygen affinity of the bloods of the fish at 15° C. where the tensions of oxygen at unloading (50% hemoglobin oxygen) are 1.4 mm. Hg for the northern brown bullhead, 12 mm. Hg for the common white sucker (1), and 17 mm. Hg for the common brook trout (2, 12). This relationship may not hold beyond the three instances given.

Results for the 16 species are presented in graphical form in Figs. 2–4. Comparative curves are given in Figs. 5 and 6.

In Figs. 2–4 the data have been averaged in groups of two or three and the averages plotted as single points. Curves for each graph were drawn freehand.

Each curve shows two portions which are significant. The first part of the curve indicates the ability of the fish to utilize oxygen at tensions of carbon dioxide which do not appear to interfere with respiration. The inflected part of the curve indicates the interference of carbon dioxide in limiting and fully preventing the utilization of oxygen despite tensions of oxygen which would be

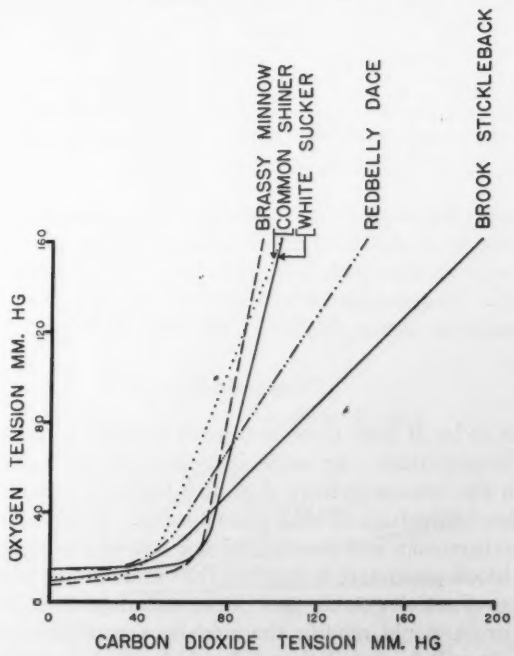

FIG. 5. Comparison of the effects of carbon dioxide on the utilization of oxygen by the brassy minnow, northern common shiner, common white sucker, northern redbelly dace, and brook stickleback.

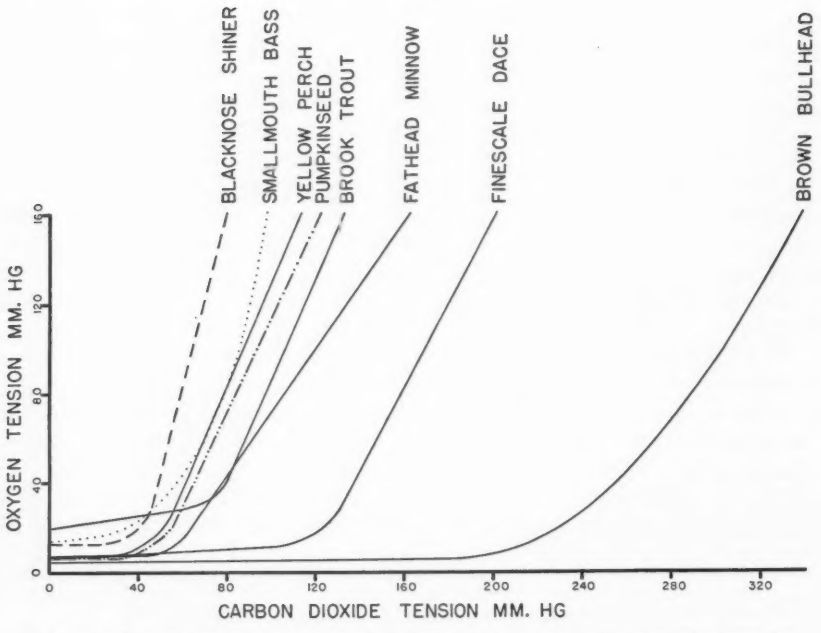

FIG. 6. Comparison of the effects of carbon dioxide on the utilization of oxygen by the northern blacknose shiner, northern smallmouth bass, yellow perch, pumpkinseed, common brook trout, northern fathead minnow, finescale dace, and northern brown bullhead.

otherwise adequate for respiration. Data in Table I summarize the tensions of oxygen remaining at death when no carbon dioxide was added, and the tension of carbon dioxide which intersects an arbitrary level of 160 mm. of oxygen at death. The species are arranged in ascending order according to the limiting tension of carbon dioxide at 160 mm. of oxygen.

Discussion

There appear to be at least three sources of variability for results at fixed conditions of temperature. In some instances at low tensions of carbon dioxide, certain fish appear to have died at a higher tension of oxygen than most of the other individuals of that same species. While neither the pH of the external environment was determined nor was any examination made of the pH of the blood plasma, it is possible that some of the variation may be due to the effects of activity on the part of the restrained fish; it is also possible that excreted urine would modify the acid–base conditions in the external environment. It will be noted that fish which are less resistant to carbon dioxide do not show an easily defined lethal level for carbon dioxide. Usually, but not always, these same species exuded great quantities of mucus.

The greatest source of variability is the effect of temperature on acclimation. Fry, Black, and Black (7) have shown that the lethal tension of carbon dioxide of goldfish acclimated at 1° C. is 60 mm. Hg, whereas fish acclimated at 32° C. have a lethal carbon dioxide tension of 200 mm. Hg. Fish at intermediate temperatures acclimated at 7°, 15°, 20°, and 25° C. showed lethal tensions of carbon dioxide of 100, 120, 140, and 170 mm. Hg, respectively. Their results showed further that reliable readings could be obtained only after complete acclimation of the fish to the temperature at which the experiments were made. However, with acclimated fish of similar stock the result could be reproduced from year to year at the same season and temperature. The effect of acclimation temperature on the lethal limit of carbon dioxide was also shown for the northern brown bullhead and the fathead minnow. However, no significant difference could be shown for the finescale dace taken in July and August, 1940.

The relationship between the effect of carbon dioxide on the blood and on the ability of the fish to extract oxygen from the water is depicted in Fig. 7 for the common sucker, carp, and northern brown bullhead. There appears then to be some relationship between the effect of carbon dioxide on the respiratory characteristics of the blood of these fishes *in vitro* and the effect of carbon dioxide upon the ability of fishes to utilize oxygen *in vivo*, for the common sucker and the northern brown bullhead. However, more than this one relationship must be considered. In the first instance, Hart (8) has shown that there are large differences in the circulatory ability of fishes. Again, inspection of the first part of the curve shows that fishes differ in their ability to withstand death in the absence of carbon dioxide. This latter difference, which must be related to the critical oxygen tension, may operate within the fish for as yet no data have been obtained on the tension of carbon

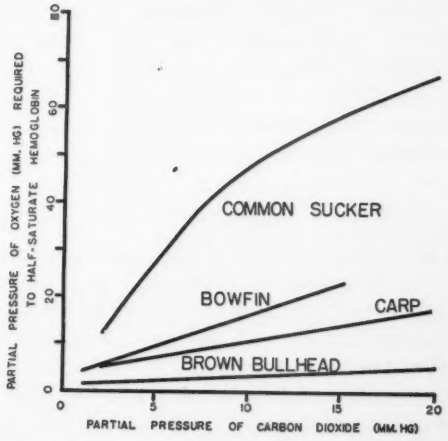

FIG. 7. Tension of oxygen necessary to half-saturate hemoglobin in whole blood of common white sucker, bowfin, carp, and northern brown bullhead at 15° C. at tensions of carbon dioxide up to 20 mm. Hg.

dioxide in the blood taken from fishes when exposed to increased levels of ambient carbon dioxide. Safford (21) observed that the carbon dioxide asphyxiation curves for the marine species tautog and toadfish were widely separated, yet the Bohr effect on the bloods is similar in magnitude (20).

Another indication that the Bohr effect and the lethal effect of carbon dioxide may not be parallel is shown in the work of Black (2) on oxygen dissociation curves on the bloods of Atlantic salmon, land-locked salmon, and brook trout acclimated to summer and winter temperature where no differences were noted for the Bohr effect. It is admitted, however, that the effect of carbon dioxide on the ability of these species to utilize oxygen in summer and winter has not been tested.

On the basis of earlier work, claims were made by Fry and Black (6) that the distribution of certain fresh-water fishes in their temperature habitat was related to the carbon dioxide sensitivity as determined by the influence of carbon dioxide on oxygen utilization. For example, the common brook trout, yellow perch, and common white sucker have similar lethal carbon dioxide curves and these species are often found in association. On the other hand, the northern brown bullhead usually inhabits warmer waters in the summer and the lethal curve shows this species to be resistant to carbon dioxide. Yet the northern creek chub and common brook trout commonly occur together in streams while the position of their lethal curves is widely separated. Consequently the generalization that the carbon dioxide sensitivity is an indicator of the temperature habitat is not tenable. Further, no satisfactory explanation can be given to account for the effect of carbon dioxide on the oxygen utilization of the intact fish. Hart (9) reported that upper lethal temperatures for a series of 10 fresh-water fishes are correlated with temperature habitats. Upper lethal temperatures and the effects of carbon dioxide on the utilization of oxygen both respond in the same directions to changes in acclimation to temperature (7, 10). Circulatory differences (8) and buffer values for whole blood (17, 18) may also influence the lethal environmental curves and help to determine the position of the fish in nature. In addition, reactions to low oxygen may operate in the distribution of fish, as indicated in the studies by Jones (13) and as discussed by Krogh (14), Fry (5), and Black (1, 3).

Summary

Sixteen species of fresh-water fishes from eight families were exposed to various concentrations of carbon dioxide above both ecological and physiological ranges. At death, when the remaining dissolved oxygen and free carbon dioxide tensions were plotted, curves were obtained which appear to be specific for a given temperature history.

When the ambient carbon dioxide tension was low, the tension of oxygen remaining in the respired water ranged from 4 mm. Hg for the northern brown bullhead to 19 mm. Hg for the common brook trout. Values for the following species ranged from 5 to 9 mm. Hg; common white sucker, northern creek chub, northern pearl dace, finescale dace, brassy minnow, northern fathead

minnow, yellow perch, and pumpkinseed. Oxygen tensions for the northern redbelly dace, northern common shiner, northern blacknose shiner, northern smallmouth bass, brook stickleback, and the eastern burbot ranged from 12 to 14 mm. Hg.

Using a tension of oxygen of 160 mm. Hg as the reference point, the limiting tension of carbon dioxide ranged from 80 mm. Hg for the northern blacknose shiner to 338 mm. Hg for the northern brown bullhead. The following species ranged from 96 to 132 mm. Hg tension of carbon dioxide: brassy minnow, 96 mm. Hg; northern smallmouth bass, 97 mm. Hg; northern common shiner, 102 mm. Hg; common white sucker, 107 mm. Hg; yellow perch and eastern burbot, 112 mm. Hg; northern fathead minnow and pumpkinseed, 122 mm. Hg; and common brook trout, 132 mm. Hg. The remaining species ranged from 182 to 234 mm. Hg carbon dioxide as follows: northern redbelly dace, 182 mm. Hg; northern pearl dace, 186 mm. Hg; brook stickleback, 193 mm. Hg; finescale dace, 202 mm. Hg; and northern creek chub, 234 mm. Hg.

Acknowledgments

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A SAMPLING SYSTEM FOR POPLAR INSECTS[1]

By W. R. Henson[2]

Abstract

The distribution of poplar foliage over the trees is described for a number of locations in the trans-Divide area of the Rocky Mountains of Canada. The statistics of some foliage attributes and of the distributions of a number of organisms infesting foliage are derived. On this basis, a sampling system with preset limits of precision and confidence is designed. The system is tested and its limitations discussed. Techniques for the modification of the system to fit local conditions are suggested. The system outlined will provide in a single operation data of known accuracy on the populations of a number of organisms.

Introduction

During the summer of 1953, a long-term study of insects inhabiting poplar in the trans-Divide region of the Rocky Mountains was begun. The project is designed to test the hypothesis (7) that insects which occupy different types of habitats may often require different climatic conditions for their optimum development, survival, and increase. A very satisfactory tree species for testing this interesting idea is trembling aspen (*Populus tremuloides* Michx.) because it supports a wide variety of insects, and provides a number of different types of insect habitats. In addition, it is widely distributed so that the investigation could be extended to many different regions after techniques have been developed and tested in the trans-Divide area, where differences in climate often occur over short distances.

The first requirement for following population fluctuations is a sampling system which will give adequate measurements of population densities. The present report deals with investigations leading to the development of such a sampling system.

Statistical Investigations on *Populus tremuloides*

General Considerations

The object of the investigations was the development of a sampling system which would provide data on the populations of insects and disease organisms inhabiting the leaves and twigs of trembling aspen. As the use to which this sampling system is to be put will probably enlarge beyond the scope of the project as it is presently envisioned, an attempt was made to place it on as broad a basis as possible. The system had to be adapted for use by a variety of field workers of varying degrees of training and skill. Therefore, it was necessary to maintain simplicity of operation even if this meant extra labor in the actual field collection of the samples.

The sampling system had to accommodate local peculiarities of stands and individual trees. Because several kinds of organisms must be sampled at the

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same time, it had to provide sufficient data on the organism presenting the most difficult sampling problem, even at the expense of oversampling more evenly distributed organisms. The system had to be designed so that the data would appear in such a form that records could be used for the purposes of a variety of special analyses. The density of the organisms sampled, as well as the nature of the comparisons to be made, fixes the manner in which field data are presented for analysis. However, for actual use in the analysis of population fluctuations, field data will probably have to be converted to some form of absolute density expression on a per tree or a per unit area basis.

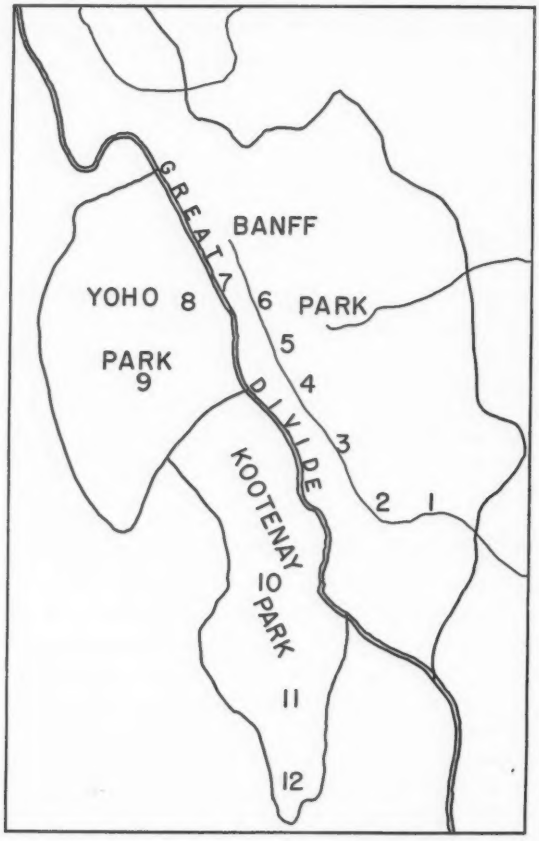

FIG. 1. The location of sampling points within the Rocky Mountain National Parks of Canada.

1	Mile 0	7	Great Divide
2	Mile 3.5	8	Cathedral
3	Mile 14	9	Leanchoil
4	Mile 19	10	Snow Creek
5	Mile 32	11	Kootenay Crossing
6	Mile 37	12	McLeod Meadows

Location, Materials, and Methods

Trees for study were selected from three areas: Kootenay, Banff, and Yoho National Parks. A number of locations within each national park were sampled, as shown in Fig. 1. Banff Park lies to the east of the Great Divide, whereas the other parks are both to the west of that barrier. Yoho Park sample locations are all within the defile of the Kicking Horse Pass, to the north of the massif formed by the Lake Louise and the Cathedral–Stephen groups of mountains. The sample locations in Kootenay Park are in the valleys of the Vermilion and Kootenay Rivers, separated from the Yoho locations by the above-mentioned massif and its subsidiaries to the south. Though both the locations in Kootenay and those in Yoho are in passes which permit the low-level passage of maritime air (2), their aspects and exposures are very different and there are indications (3) that their climates show corresponding differences.

The distribution of the sample trees is given in Table I. The sampling was concentrated at Kootenay Crossing. This was done because time did not permit widespread intensive sampling, and the Kootenay Crossing stand was selected as both suitable and typical. Therefore in the following discussion, the results will be presented first for this stand. The characteristics of the attribute under discussion will be derived for the Kootenay Crossing stand and used as a basis of comparison for the single tree samples taken from other locations and other areas.

Trees of *Populus tremuloides* were selected randomly within each location. Each tree was felled, its total height recorded, and the orientation and arrangement of its various segments were marked. Thereafter it was cut into smaller parts for easier handling. Branches were treated individually. Records

TABLE I

THE DISTRIBUTION OF SAMPLE TREES BY AREA AND LOCATION

Area	Location	Number of trees	
Kootenay	Snow Creek	1	
	Kootenay Crossing	18	
	McLéod Meadows	1	
			20
Banff	Mile 0	2	
	Mile 3.5	1	
	Mile 14	1	
	Mile 19	1	
	Mile 32	1	
	Mile 37	1	
			7
Yoho	Great Divide	1	
	Cathedral	1	
	Leanchoil	1	
			3
			30

were made to show (*a*) the height and orientation of the branch; (*b*) the location of each leaf bunch* as numbered from the trunk to the periphery; (*c*) the number of leaves in each bunch; and (*d*) the number of organisms on each leaf and the total for the bunch. These data were recorded in such a way that totals could be obtained rapidly, but the original data on leaf populations could be referred to as required.

The crown of the tree was divided on the basis of height into lower, middle, and upper thirds. In addition, terminals were kept separate in the first analysis. The horizontal classes were the four cardinal compass quadrants. The first problem was to determine the sampling unit. It was felt that if a leaf bunch could be used, the recording of the data could be simplified.

Number of Leaves in the Bunch

The mean number of leaves per bunch was derived for each of the 16 segments of each tree examined. The results were subjected to the analysis of variance on a single tree basis with the height in the crown plus terminals (four classes) and the compass direction (four classes) forming the basis of a four-by-four, single-level analysis. The over-all mean number of leaves per bunch is 4.562 ± 0.266.

No persistent trend in the number of leaves per bunch could be detected within the tree for level or direction, nor could a trend be detected between trees, locations, or areas in the distribution of number of leaves per bunch. Thus, the leaf bunch may be considered to have substantially the same number of leaves regardless of level within the tree, direction in the crown, tree within the location, location within area or between areas.

The variance ratio for level was of border significance ($F = 3.87 : 5\% = 3.86$). This ratio is apparently due to the number of leaves in the terminal bunches. The absolute amount of foliage which is contained in the terminal bunches, however, is very small. The discarding of the leaves in the terminal bunches did not affect the size of the total sampling universe ($\chi^2 = 0.00709$ for degrees of freedom 47/11) and the inclusion of the terminal bunches with those of the upper crown class did not affect the size of the upper crown with respect to the whole ($\chi^2 = 0.00659$ for degrees of freedom 36/47). Therefore, the terminal tissue was lumped with that of the upper crown, since it was not considered advisable to discard it because of its possible ecological significance. When this was done, the variance ratio for level was reduced considerably ($F = 2.56 : 5\% = 4.07$).

Distribution of Leaf Bunches

As the leaf bunch is remarkably constant, it is possible to base the examination of foliage distribution and the distribution of organisms on the foliage on the number of bunches and density per bunch instead of on individual leaves. This reduces the labor of recording in the field and speeds calculation.

* *The foliage of poplar is arranged in bunches of leaves growing from the terminal of the current year's growth. The bunches which are lateral to the main branches are compact, those which are terminal are diffuse.*

The trees of the Kootenay Crossing location were analyzed separately. Differences in the numbers of bunches due to direction in the crown were uniformly insignificant. Differences due to crown level showed intermittent significance. However, when all trees from the location were analyzed together with the means for crown segments used in the analysis, the level in the crown was shown to be significant. A summary of the analysis is given in Table II.

TABLE II

THE ANALYSIS OF NUMBERS OF LEAF BUNCHES FOR DIRECTION AND HEIGHT IN THE CROWN—MEANS OF KOOTENAY CROSSING TREES

Source	Degrees of freedom	Mean squares	*F*
Total	15	1221.78	
Level	3	5307.74	3.89*
Direction	3	413.66	
Error	9	1362.50	

The significance of level persisted when the analysis was extended to include trees from other sources. Table III gives a summary of such an analysis which included all trees taken from the area of Kootenay Park.

TABLE III

THE ANALYSIS OF NUMBERS OF LEAF BUNCHES FOR DIRECTION AND FOR CROWN LEVEL—MEANS OF TREES FROM THREE KOOTENAY PARK LOCATIONS

Source	Degrees of freedom	Mean squares	*F*
Total	47	6590	
Level	3	43371	5.40**
Direction	3	864	
Error	41	8028	

A similar situation may be demonstrated for the trees from Banff Park. Table IV presents a summary of the analysis for the mean Banff Park values.

TABLE IV

THE ANALYSIS OF NUMBERS OF LEAF BUNCHES FOR DIRECTION AND FOR CROWN LEVEL—MEANS OF TREES FROM SIX BANFF PARK LOCATIONS

Source	Degrees of freedom	Mean squares	*F*
Total	111	13146	
Level	3	91546	6.10**
Direction	3	32780	
Error	106	14998	

A slightly lower level of significance is shown in the trees from Yoho Park. However, the general distribution is the same as that demonstrated from the other two areas. The summary of the analysis is given in Table V.

TABLE V

THE ANALYSIS OF NUMBERS OF LEAF BUNCHES FOR DIRECTION AND FOR CROWN LEVEL—MEANS FOR TREES FROM THREE YOHO PARK LOCATIONS

Source	Degrees of freedom	Mean squares	*F*
Total	47	1908	
Level	3	8244	3.25*
Direction	3	2676	
Error	41	2533	

It may be demonstrated that the distribution of the leaves is similar in all three parks. A summary of such an analysis is presented in Table VI.

TABLE VI

THE ANALYSIS OF NUMBERS OF LEAF BUNCHES FOR DIRECTION AND LEVEL WITHIN CROWN AND FOR AREA—MEANS OF TREES FROM KOOTENAY, BANFF, AND YOHO PARK

Source	Degrees of freedom	Mean squares	*F*
Total	47	3084.97	
Level	3	21652.86	3.87*
Direction	3	8052.80	
Area	31	220.07	
Error	10	5587.67	

The homogeneity of the distributions for the three parks was also tested by a comparison of the residual error variances which were calculated from the mean values from each park. The comparison was made by means of the *F* ratio. It was found that the residual variances from all three parks were of similar order. This is an indication that there is no reason to suspect a lack of homogeneity between the populations represented by the trees of the three parks.

Summary of the Analysis of Foliage

It has been shown that the number of leaves in each bunch on the trees examined from Kootenay, Banff, and Yoho National Parks is remarkably constant with respect to position in the crown, location, and area. Because of this consistency, the number of bunches rather than the number of leaves was used as a measure of the amount of foliage. The mean number of leaves per bunch was 4.562 ± 0.266.

On this basis, it has been shown that the distribution of foliage of poplar in Kootenay, Banff, and Yoho Parks is not significantly variable with respect to its compass direction within the tree, location, or area of origin. However, there is a significant difference in the amount of foliage at various heights in the crowns of the trees.

For sampling purposes, therefore, foliage may be taken at random between trees and with respect to direction within trees at any location. However, the sample must be stratified with respect to level within the crown. This is best done (4) by distributing the sample in the same ratio as is exhibited by the variances calculated for levels in the foregoing analysis. The variances for levels are: lower 112993.58, middle 68012.44, and upper, 39436.47. Roughly, this ratio is 3 : 2 : 1. Therefore, a sample which is taken at random between trees and between directions within the trees, and stratified within the trees in the ratio of lower, 3: middle, 2: upper, 1, with the required number of bunches being taken within each level, will yield a true representation of the trees from the location sampled. The number of bunches required will be established in a later section of this paper after the discussion of insect distributions and other subsidiary points.

Leaf Size

Early in the examination of the foliage from different locations, it was noted that the size of the leaves showed wide variation. At high insect densities, the amount of leaf tissue rather than the number of leaves may well be a more adequate measurement of the substrate. Therefore, the possible need for a correction for leaf size was investigated.

Leaves, taken at random within trees and between trees for each location, were brought to the field station. The outlines of 100 leaves from each location were traced on paper. The area of each leaf was measured with a planimeter and the measurements were checked by direct graphic determination. In addition, the length and maximum width of each leaf was measured. It was shown that the mean leaf size did vary with location. However, the leaf size was remarkably constant within a location. Three locations were selected for further testing. A summary of these data is presented in Table VII.

TABLE VII

A SUMMARY OF LEAF MEASUREMENTS FROM GREAT DIVIDE, KICKING HORSE PASS, AND HERBERT LAKE (MEASUREMENTS IN CM.)

Location	Mean area, $cm.^2$	Mean length, cm.	Mean width, cm.
Great Divide	7.39 ± 0.57	3.71 ± 0.06	3.12 ± 0.03
Kicking Horse Pass	15.14 ± 0.41	5.16 ± 1.17	4.35 ± 0.11
Herbert Lake	6.69 ± 0.65	3.20 ± 0.05	3.12 ± 0.06

Regressions were calculated for the relationships between length, width, and area of leaf for each location. Simple correlations were calculated for the same relationships and the values were compared between locations. The values found for the correlations were extremely high for all locations with the over-all coefficient of correlation between area and length of leaf being 0.9856 (for 300 pairs of values). The coefficient between area of leaf and leaf width was 0.9884 (for 300 pairs of values).

The shape of the leaves varied to some extent as shown by a slight variation in the regression coefficients between length and width and area at the various locations. However, these variations were not significant (pooled $\chi^2 = 0.359 : 5\% = 1.635$) and the over-all regression value of 0.4166 was an adequate approximation of the individual regressions for area on length. Since it is easier to measure length of leaf than to measure width in the field, the length should be used as an index of area.

In order to correct for leaf size so that population data may be directly compared between locations where the sizes of leaves are different, the mean length of 100 leaves taken at random should be determined. Division of this mean leaf length by 0.4166 will give a value for the mean leaf area. This, multiplied by the number of leaves sampled (number of bunches times number of leaves per bunch), may be used to convert the population figure for the location from number of organisms per standard number of leaf bunches to number for an aggregate leaf area. The resulting datum could conveniently be reduced to number of organisms for a standard leaf area. An example of the application of such a correction will appear in the section which deals with the actual sampling technique.

Organisms Infesting Aspen

During the recording of the data on foliage distribution, a record was maintained of the insects and diseases infesting the leaves and terminal twigs of the trees. Identifications were not possible in the field, so the organisms were simply listed by appropriate descriptive names. The present identifications are all tentative as most of them are based on immature forms. When complete series of material have been obtained, positive identifications will be possible.

As recorded in the field, the population data were in the form of a simple record of the number of organisms on each leaf bunch. This number was then broken down in the field records into the number of organisms on each leaf of the bunch. This was done at the time because the utility of the leaf bunch had not been established as a sampling unit. The present analysis was done on the basis of number of organisms per thousand bunches. This conversion was done in order to permit easy calculation and comparison of results. The data on infestation of individual leaves may well be of considerable biological interest. However, from the viewpoint of the sampling problem, it is simpler to use the larger unit of the leaf bunch rather than the individual leaf.

The sampling technique must be based on an understanding of the relationship between foliage and insect distributions. Therefore, no corrections for leaf size were applied for the purposes of this preliminary analysis.

It was shown that all but two of the organisms infesting aspen were distributed on the tree in the same manner as the foliage. That is, the density of infection showed no variation with either level or direction in the crown.

Slight regional differences from this general finding were detected. However, these differences were not significant in the over-all distribution of the organisms involved. Differences between locality and area densities which were detected were due to over-all density variations and not to changes in the patterns of distribution within the tree. This point was established by the finding that the interactions between level, and direction in the crown, and locality were in no case significant.

Two organisms were found to show preferences for one side of the tree. For sampling purposes, it was decided to oversample sufficiently that these directional differences in density could be ignored in the sample design. Of course, such oversampling would not result in an error in the figure obtained for population since the final expression of the population is to be in terms of over-all density.

The Design of the Sample

Because of the considerations discussed above, samples of poplar foliage taken in order to determine the densities of the organisms studied should be distributed in the same manner as the foliage within the tree. The largest sample is required for organisms that show the largest proportion of unexplained variance with respect to position in the tree. As the error term of the variance analyses which were carried out on organism distributions is made up of all unassigned and residual variance, the ratio of mean squares for error to total mean squares may be used as an index of the amount of unexplained variance in the distribution of each organism. A list of such indices for each organism studied is given in Table VIII.

TABLE VIII

THE INDEX OF UNEXPLAINED VARIANCE FOR EACH ORGANISM IN THE SAMPLING STUDY

Organism		Index of unexplained variance
Leaf Spot	(*Septoria musiva* Pk.)	0.75
Black Spot	(*Sclerotinia whetzelii* Seaver)	0.61
Aspen Tortrix	(*Archips conflictana* Wlkr.)	0.49
Leaf Beetle	(*Phylodecta americana* Schffr.)	0.44
Leaf Miner	(*Phyllocnistis populiella* Chamb.)	0.42
Aphid 1		0.37
Aphid 2		0.36
Stem Gall		0.36
Petiole Gall 1		0.31
Taphrina	(*Taphrina aurea* Pers.)	0.30
Petiole Gall 2		0.29
Edge Gall		0.28
Pit Gall		0.28

All the identifications given in Table VIII are tentative. No identifications are given for the aphids or the galls because no mature forms have been recovered yet. Descriptive names are given simply for ease of reference.

The largest amount of unexplained variance is found in the distribution of leaf spot. This might be expected because this organism displays the distribution which varies most markedly from that of the foliage. A sampling system which will yield sufficient data for leaf spot will automatically yield sufficient data (within the same limits) for all other organisms included in this study.

Though the distributions of leaf spot (and black spot) are different from that of the foliage, it was considered advantageous to distribute the sample according to the foliage pattern. Such a sample distribution imposes oversampling in a segment of the tree but this is acceptable for the sake of simplicity. The advantage of such a technique is that it makes possible the detection of position anomalies.

The size of the sample required to give sufficient data for leaf spot within 99% confidence at a precision of $\pm$ 5% was calculated from the formula given by Oakland (5). The formula is:

$$n = 2t^2 pq/D^2$$

where t is the normal deviate corresponding to the level of significance in a two-tailed test (1), D is the confidence expressed as a decimal, p is the probability of the event, and $q = 1-p$.

In this case, a value for p was derived from the known over-all density of the organism: $p = 0.2959$ (based on the probability of an individual leaf bearing a single spot). With this value of p, the sample size required was calculated to be 1102 leaves. The equivalent number of bunches was 240.87.

A number of preliminary test samples were taken with the aid of random number tables (6) from the field data. These samples all contained 240 leaf bunches stratified between the crown levels in the ratio of lower, 3: middle, 2: upper, 1, and at random with respect to direction within the crown. It was found that the best precision was obtained in these preliminary samples when the leaf bunches were taken in equal numbers from 20 trees.

A further series of 10 test samples was taken with the aid of random number tables. Each sample contained 240 leaf bunches distributed as 12 bunches from each of 20 trees, taken at random with respect to direction in the crown, and in the ratio of 3 : 2 : 1 between the lower, middle, and upper thirds of the crown.

The test samples all yielded figures for leaf spot density which were within acceptable limits of the true density found by complete counts of the trees. Pooled χ^2 was used to test the homogeneity of the samples and the result ($p = 0.99 +$) showed that the sample results were satisfactory.

The density of black spot, the organism which showed the second greatest diversity, was calculated from the same test samples in order to test the

applicability of the system to the sampling of other organisms. The samples yielded figures for black spot density which were both homogeneous and within acceptable limits of the true density ($p = 0.99 +$).

Discussion

A sampling system has been designed for a series of organisms which depends for the reliability of its results on the distributions of the organisms over the crowns of the infested trees.

The system was designed on the basis of data taken during one season only. Therefore, the distributions of the organisms are known for only one density and one climatic season. It is possible that at different densities, or under different climatic conditions, the organisms will not be distributed in the same manner. In addition, if the density of one of the various organisms increases to the point where considerable defoliation results, the distribution of the foliage of the tree might well be distorted. If this were to happen, the technique of sampling and also the manner in which the results were stated would have to be modified. Some expression of absolute density would be required.

Because of these factors, it would be unwise to assume that the size and distribution of the sample which has been derived in this contribution would invariably give accurate results. However, the system may be tested and, if necessary, modified to suit field conditions. Testing should be done on the basis of complete counts of foliage and organisms on a series of trees. The number of trees which would have to be examined in this way would depend on whether or not it was found that the patterns in the first few differed from those described herein. However, the field counts could be held to a minimum by a continuing analysis of the results which would allow the counts to be stopped as soon as the statistical requirements have been met.

A new sample could be designed in the manner set forth in this paper which would meet the requirements of a changed situation. Because the precision and confidence of the sample are set during the process of sample design, the results of sampling, even if the sample had to be redesigned from time to time, would be directly comparable. This condition would be met because any departure from the imposed limits would be in the direction of increased precision.

The Expression of Sampling Results

If a figure for absolute density is required for the estimation of the total population of the organism, the sampling data may be expressed in terms of number per tree, or, better still, number per acre. In order to convert to such terms, it would be necessary to derive a technique for the estimation of the amount of foliage in an acre. Such a determination would have to be made for each stand investigated. This aspect of the problem has not been examined.

For the purposes of the design of a sampling system, all figures on populations are considered in terms of density. For many purposes, such an expression is far from adequate. The amount of foliage which supports the organisms may show wide variation when it is measured only in terms of the number of leaves or the number of leaf bunches. So, even expressions of populations in terms of density per leaf bunch are open to considerable error.

In order to permit comparisons of density between localities, the figures must be based on comparable segments of the substrate. In this case, the most important source of variation is the size of the leaf.

It has been shown that a very close estimate of mean leaf size can be obtained from a measurement of mean leaf length. If figures for insect density are required, the expression of density per unit leaf area is probably the most satisfactory. The conversion from field data presents no difficulty.

For example, suppose a density of 200 organisms per 1000 leaf bunches has been found from field sampling. A measurement would be made of the length of 100 leaves, taken at random within the original sample. The mean length of the leaves would be calculated. Suppose this were 3.00 cm. The mean leaf area would be $3.00/0.4166 = 7.2011$ cm.^{2} (where 0.4166 is the coefficient of regression of leaf length on leaf area). From this, the density of the organism would be: $200 \times 10{,}000/7.201 \times 4.562 \times 1000 = 60.881$ organisms per 10,000 cm.^{2} leaf surface (where mean leaf area is 7.201 cm.^{2} and mean number of leaves per bunch is 4.562). The unit area, 10,000 cm.^{2}, is chosen because it is a convenient figure in the same order of magnitude as the area of leaf surface per 1000 leaf bunches.

Summary

1. The foliage of *Populus tremuloides* Michx. appears as a remarkably constant number of leaves on each twig of the current year's growth. The statistics of these bunches of leaves are derived, and it is shown that the number of leaf bunches may be used as a measure of the number of leaves.

2. The distribution of foliage over the crown of poplar is described.

3. The characteristics of leaf area, length, and width are derived and a technique is described for correcting data based on leaf number, for size of leaf.

4. The distributions of a number of organisms infesting poplar are discussed. A comparison of the amount of unassigned variance in these distributions is made in order to detect the most variant organism for sampling purposes.

5. A sampling system with preimposed limits of precision and confidence is designed on the basis of the distribution of the foliage and of the most variant organism with respect to foliage.

6. The sampling system is tested both for the organism for which it was designed, and for another organism. It is shown that satisfactory figures for density for all organisms included in the study may be obtained in one sampling operation.

7. Various methods of expressing population data and the use of a correction factor for leaf size are discussed.

8. The possible need for continued modification of the sampling system to take into account changes in field conditions is discussed. The technique for such examination and for redesigning the sample is outlined.

Acknowledgments

The writer would like to express his appreciation of W. G. Wellington's direction and critical review of the work. G. B. Oakland suggested many of the statistical techniques and reviewed the contribution. Field assistance was given by R. F. Shepherd, and L. C. Weir assisted with the bulk of the computational work. W. C. McGuffin and V. J. Nordin helped with tentative identifications of field material.

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CORRECTION

Page 329. Couplet 4 of the "Key to the *Aedes* Black-legged Complex in the Churchill, Manitoba, Region" should read:

4. Post procoxal scale patch present; tarsal claw resembling that of *A. impiger*..*A. implicatus*

If posterior portion of probasisternum rubbed bare of scales and tarsal claw resembles that of *A. nigripes*.....*A. punctor*

Post procoxal scale patch absent.....5

Notes to Contributors

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Manuscripts should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables (each typed on a separate sheet) and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

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An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

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